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Please see brief summary of full prescribing information on next page.

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(7 orange tablets containing inert ingredients are included in the 28-day regimen.)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

> **WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.**
>
> The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk. C—Use associated with moderate risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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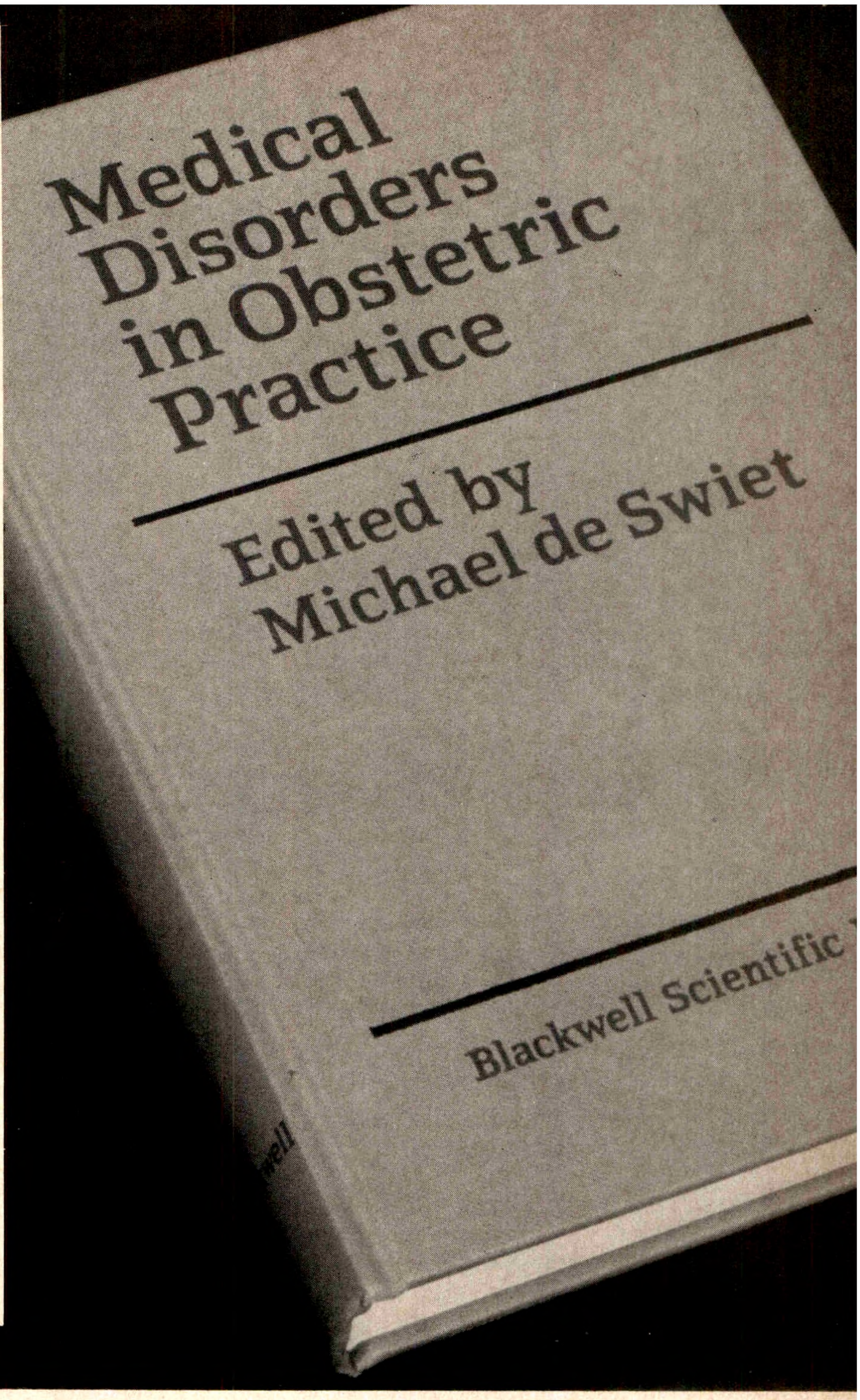
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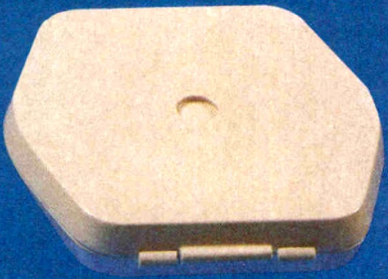

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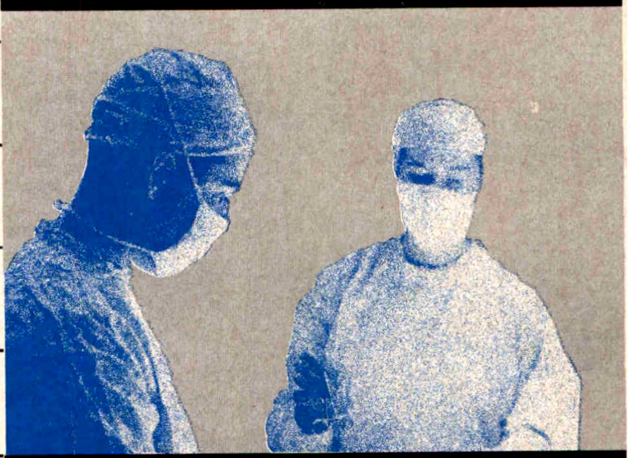

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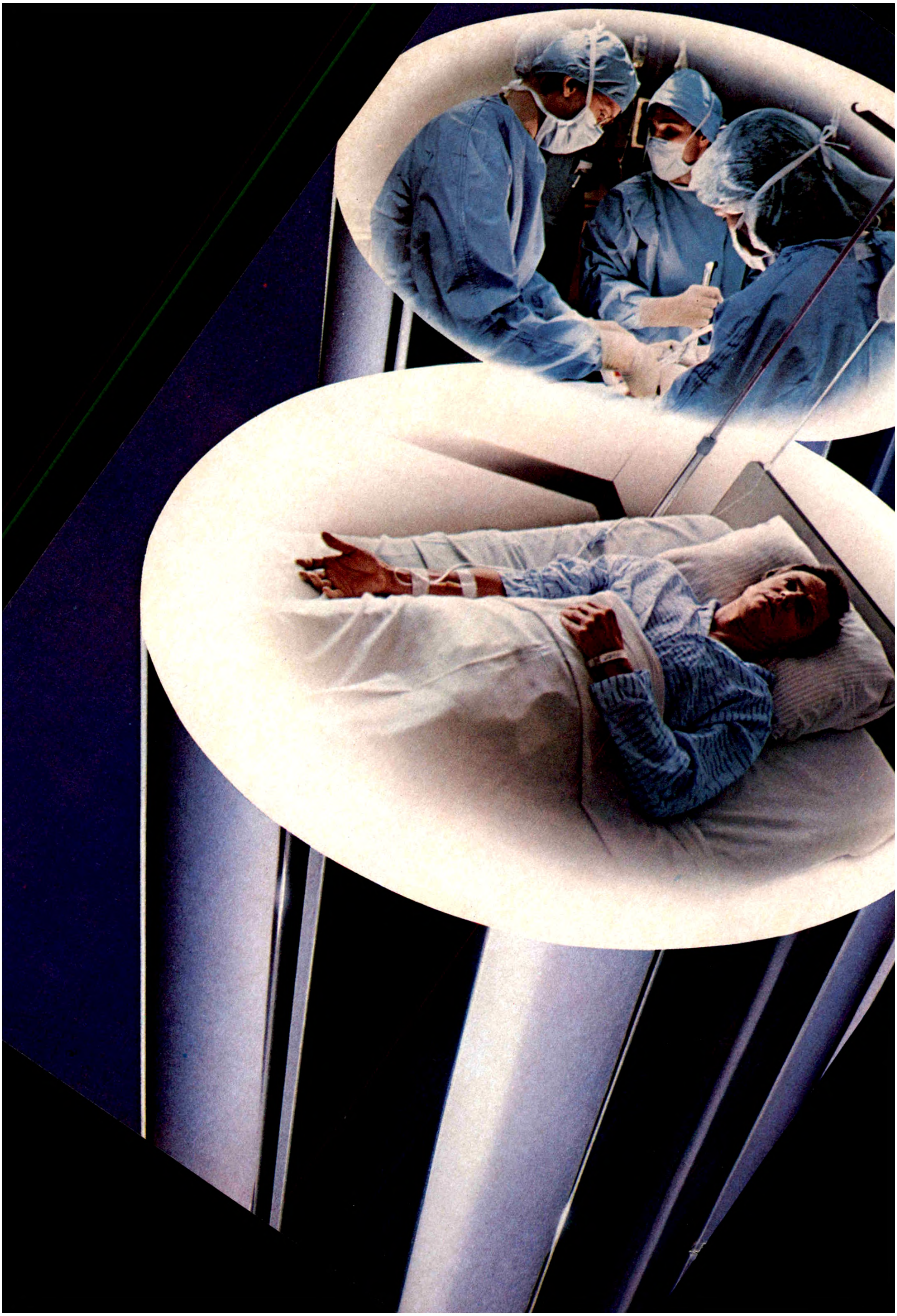

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[†] In infections due to susceptible strains of indicated organisms.

[‡] *In vitro* susceptibility does not necessarily imply a correlation with *in vivo* results.

[§] Based on PDR, 1984; Manufacturer's Prescribing Information.

[¶] In severe infections, or infections caused by less sensitive organisms, the total daily dose and/or frequency *may be* increased. Patients have been successfully treated with a total daily dosage of 6 to 12 grams divided into two, three, or four administrations ranging from 1.5 to 4 grams per dose.

[1] Carlin HS, Romankiewicz J: The impact of dosage regimen on intravenous drug cost. Scientific Exhibit, American Society of Hospital Pharmacists, New Orleans, Dec 6-10, 1981.

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PRECAUTIONS: Although transient elevations of the BUN and serum creatinine have been observed, CEFOBID alone does not appear to cause significant nephrotoxicity However, concomitant administration of aminoglycosides and other cephalosporins has caused nephrotoxicity.

CEFOBID is extensively excreted in bile. The serum half-life of CEFOBID is increased 2–4 fold in patients with hepatic disease and/or biliary obstruction. In general, total daily dosage above 4 g should not be necessary in such patients If higher dosages are used, serum concentrations should be monitored.

Because renal excretion is not the main route of elimination of CEFOBID (see CLINICAL PHARMACOLOGY), patients with renal failure require no adjustment in dosage when usual doses are administered. When high doses of CEFOBID are used, concentrations of drug in the serum should be monitored periodically. If evidence of accumulation exists, dosage should be decreased accordingly.

The half-life of CEFOBID is reduced slightly during hemodialysis. Thus, dosing should be scheduled to follow a dialysis period. In patients with both hepatic dysfunction and significant renal disease, CEFOBID dosage should not exceed 1–2 g daily without close monitoring of serum concentrations.

As with other antibiotics, vitamin K deficiency has occurred rarely in patients treated with CEFOBID Those at risk include patients with a poor nutritional status, malabsorption states (e.g., cystic fibrosis), alcoholism, and patients on prolonged hyper-alimentation regimens (administered either intravenously or via a naso-gastric tube). Prothrombin time should be monitored in these patients and exogenous vitamin K administered as indicated.

A disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia has been reported when alcohol (beer, wine) was ingested within 72 hours after CEFOBID administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOBID A similar reaction has been reported with other cephalosporins.

Prolonged use of CEFOBID may result in the overgrowth of nonsusceptible organisms. Careful observation of the patient is essential.

CEFOBID should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Drug Laboratory Test Interactions: A false-positive reaction for glucose in the urine may occur with Benedict's or Fehling's solution.

Carcinogenesis, Mutagenesis, Impairment of Fertility: The maximum duration of CEFOBID animal toxicity studies is six months. In none of the *in vivo* or *in vitro* genetic toxicology studies did CEFOBID show any mutagenic potential at either the chromosomal or subchromosomal level. CEFOBID produced no impairment of fertility and had no effects on general reproductive performance or fetal development when administered subcutaneously at daily doses up to 500 to 1000 mg/kg prior to and during mating, and to pregnant female rats during gestation. These doses are 10 to 20 times the estimated usual single clinical dose.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in mice, rats, and monkeys at doses up to 10 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to CEFOBID. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Only low concentrations of CEFOBID are excreted in human milk. Although CEFOBID passes poorly into breast milk of nursing mothers, caution should be exercised when CEFOBID is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: In clinical studies the following adverse effects were observed and were considered to be related to CEFOBID therapy or uncertain etiology:

Hypersensitivity: As with all cephalosporins, hypersensitivity manifested by skin reactions (1 patient in 45), drug fever (1 in 260), or a change in Coombs' test (1 in 60) has been reported. These reactions are more likely to occur in patients with a history of allergies, particularly to penicillin.

Hematology: As with other beta-lactam antibiotics, reversible neutropenia may occur with prolonged administration. Slight decreases in neutrophil count (1 patient in 50) have been reported. Decreased hemoglobins (1 in 20) or hematocrits (1 in 20) have been reported, which is consistent with published literature on other cephalosporins. Transient eosinophilia has occurred in 1 patient in 10.

Hepatic: Of 1285 patients treated with cefoperazone in clinical trials one patient with a history of liver disease developed significantly elevated liver function enzymes during CEFOBID therapy. Clinical signs and symptoms of nonspecific hepatitis accompanied these increases. After CEFOBID therapy was discontinued, the patient's enzymes returned to pre-treatment levels and the symptomatology resolved. As with other antibiotics that achieve high bile levels, mild transient elevations of liver function enzymes have been observed in 5–10% of the patients receiving CEFOBID therapy. The relevance of these findings, which were not accompanied by overt signs or symptoms of hepatic dysfunction, has not been established.

Gastrointestinal: Diarrhea or loose stools has been reported in 1 in 30 patients. Most of these experiences have been mild or moderate in severity and self-limiting in nature. In all cases, these symptoms responded to symptomatic therapy or ceased when cefoperazone therapy was stopped. Nausea and vomiting have been reported rarely.

Symptoms of pseudomembranous colitis can appear during or for several weeks subsequent to antibiotic therapy (see WARNINGS).

Renal Function Tests: Transient elevations of the BUN (1 in 16) and serum creatinine (1 in 48) have been noted.

Local Reactions: CEFOBID is well tolerated following intramuscular administration. Occasionally, transient pain (1 in 140) may follow administration by this route. When CEFOBID is administered by intravenous infusion some patients may develop phlebitis (1 in 120) at the infusion site.

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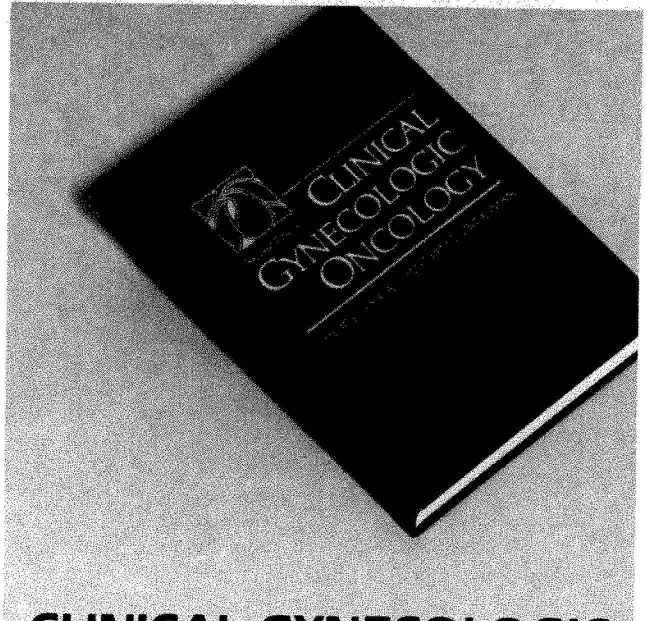

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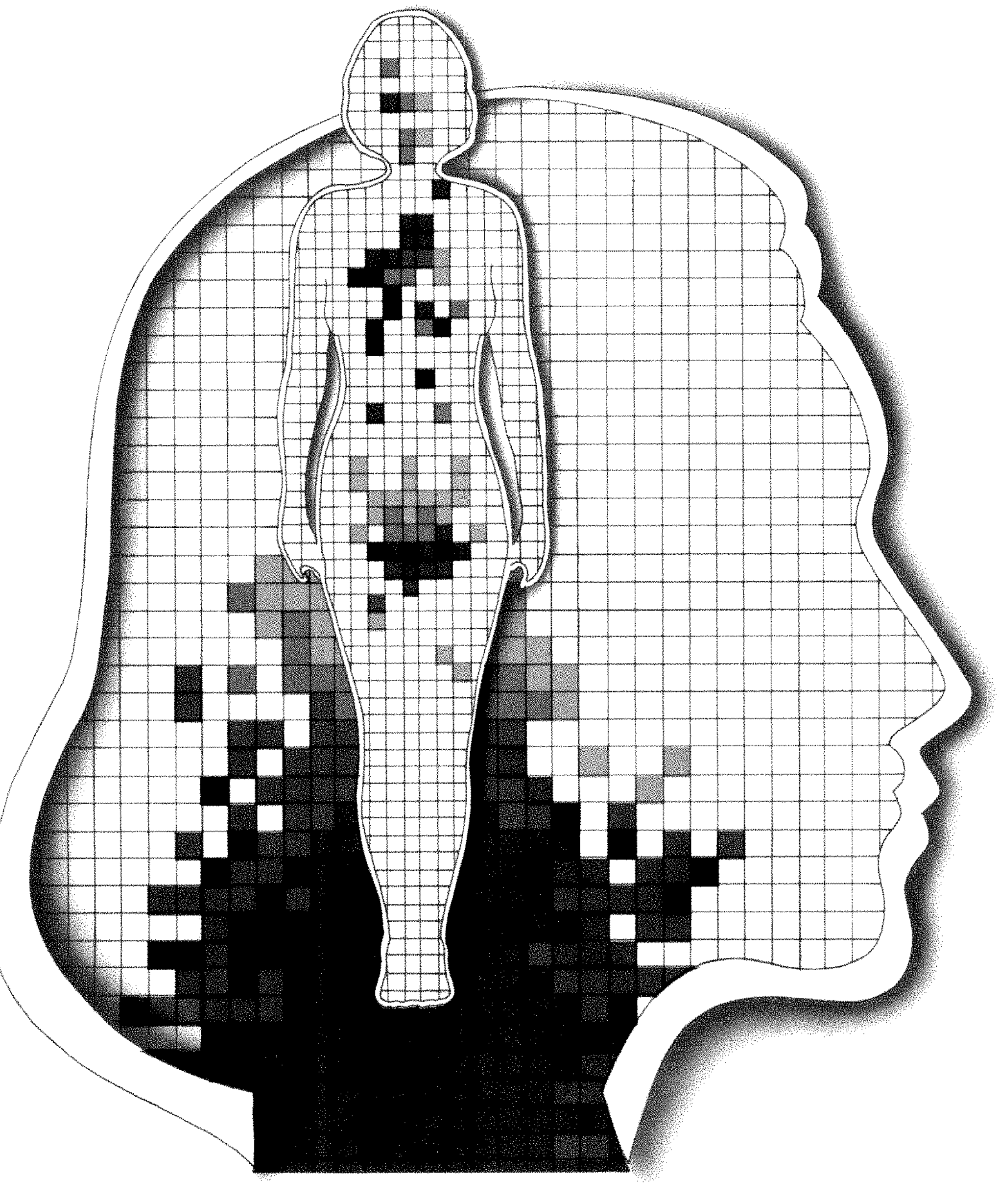

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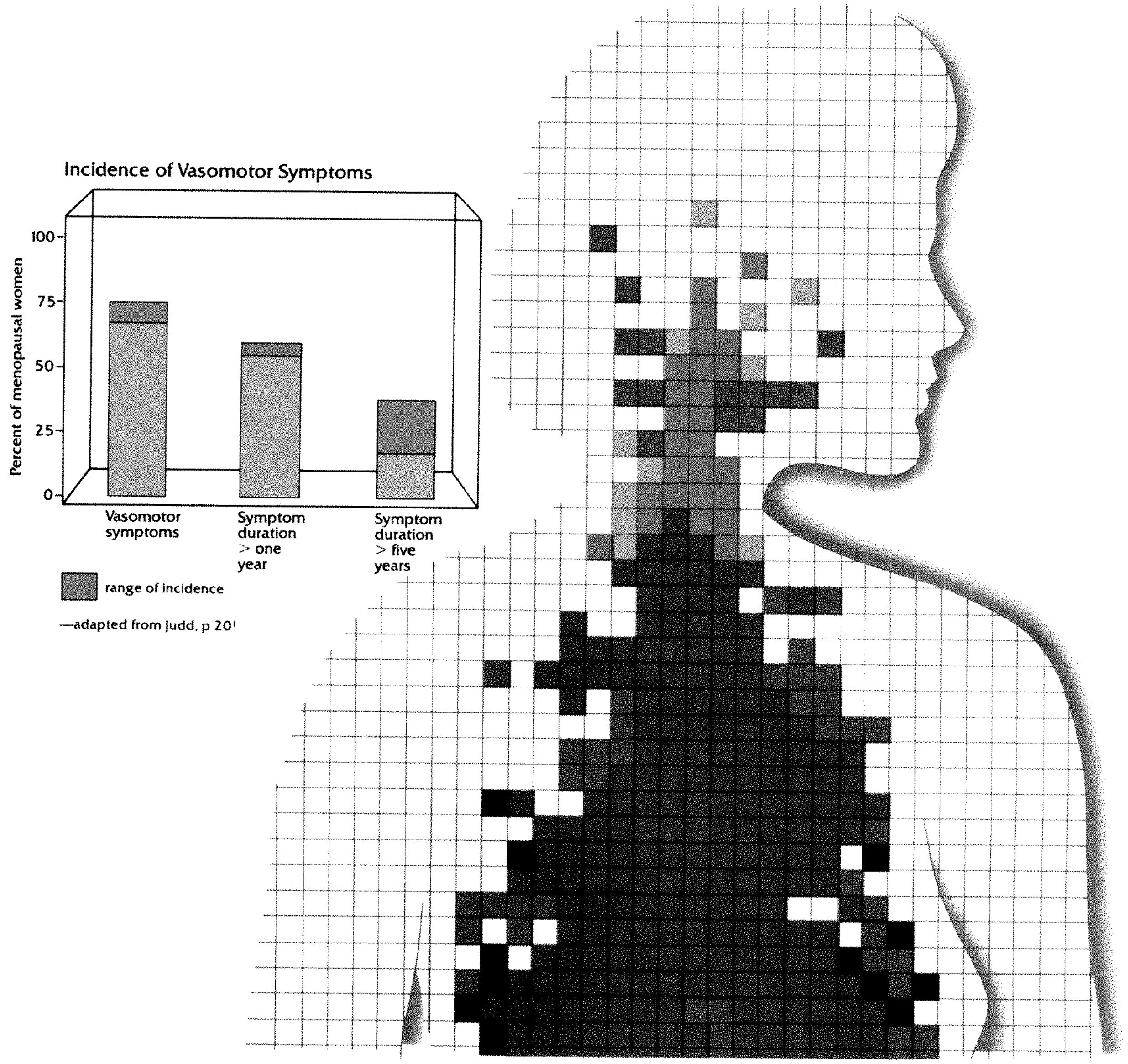
Three profiles

VASOMOTOR SYMPTOMS THAT DEMAND INTERVENTION



—adapted from Judd, p 20[1]

PREMARIN RELIEVES MODERATE TO SEVERE VASOMOTOR SYMPTOMS

Vasomotor symptoms are the most common manifestation of the menopause, affecting up to 75% of menopausal women. Of these, 80% may suffer for more than a year and up to 50% for more than five years.[1] These symptoms can disrupt a woman's life by chronically interrupting sleep, resulting in anxiety and irritability.

In a study of postmenopausal women suffering severe episodes of cutaneous flushing, symptoms improved markedly after administration of estrogen[2]—the treatment of choice for moderate to severe vasomotor symptoms.[3] The estrogen of choice is PREMARIN, the most widely prescribed estrogen for over 40 years. PREMARIN (Conjugated Estrogens Tablets, U.S.P.) relieves moderate to severe vasomotor symptoms of the natural menopause, as well as the acute and often severe symptoms of surgical menopause.

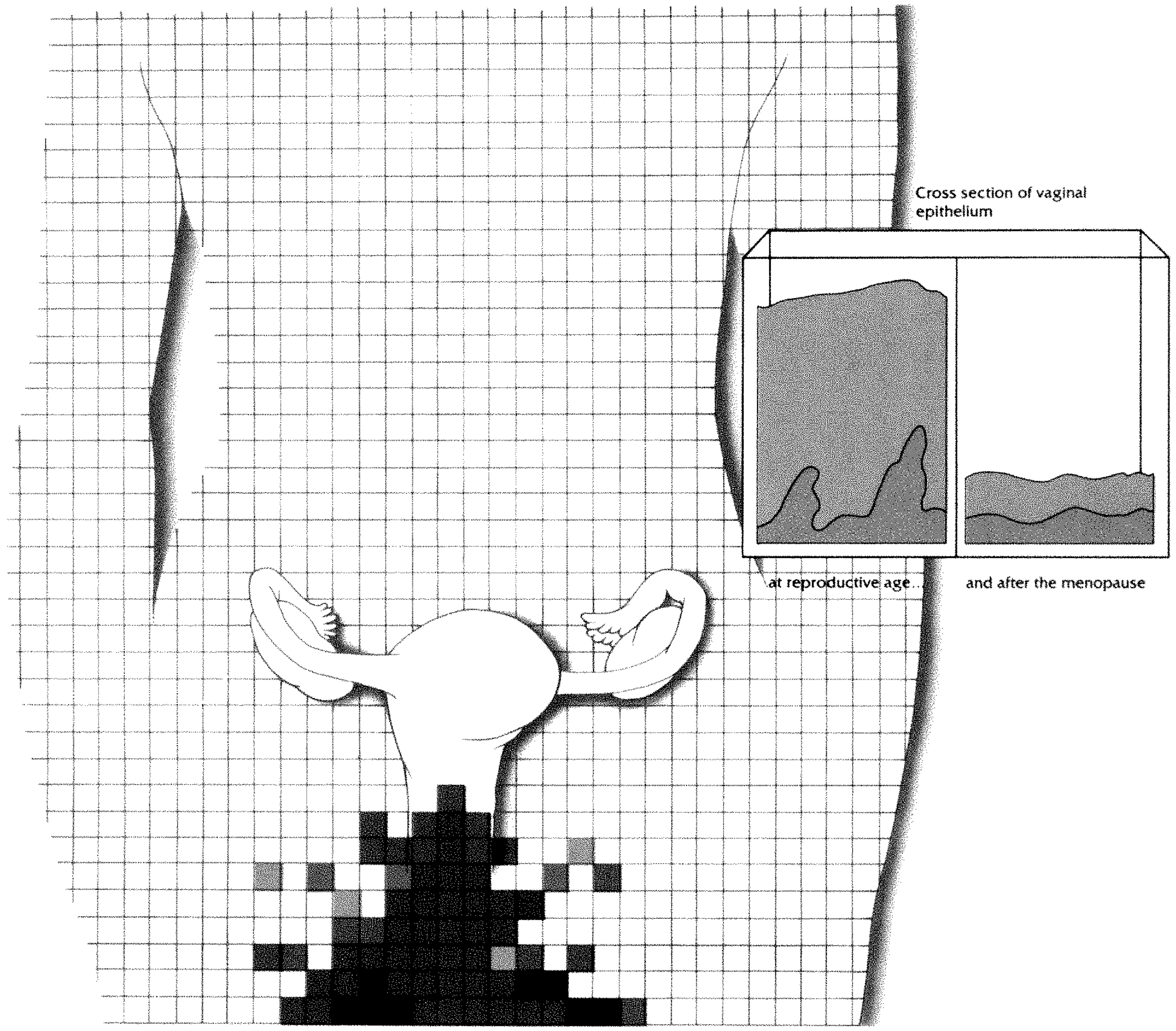
PREMARIN®

(CONJUGATED ESTROGENS TABLETS, U.S.P.)

0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

Please see last page for brief summary of full prescribing information.

VAGINAL ATROPHY THAT INTERFERES WITH SEXUALITY



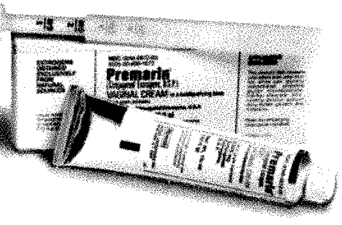
PREMARIN RESTORES THE VAGINAL ENVIRONMENT

In the postmenopausal woman, decreasing levels of estrogen can have devastating effects on a woman's sexual functioning. The pH of vaginal secretions rises, promoting the growth of contaminating organisms. The vaginal epithelium dries and thins, becoming susceptible to irritation, injury, and infection. Sexual relations may be difficult or impossible.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream focuses therapy at the site of the problem. Vaginal dryness is relieved, pH reverts to its normal acidity, and the epithelium thickens and becomes more resistant to injury and infection. With the vaginal environment returned to its premenopausal state, sexual function may improve.

PREMARIN®

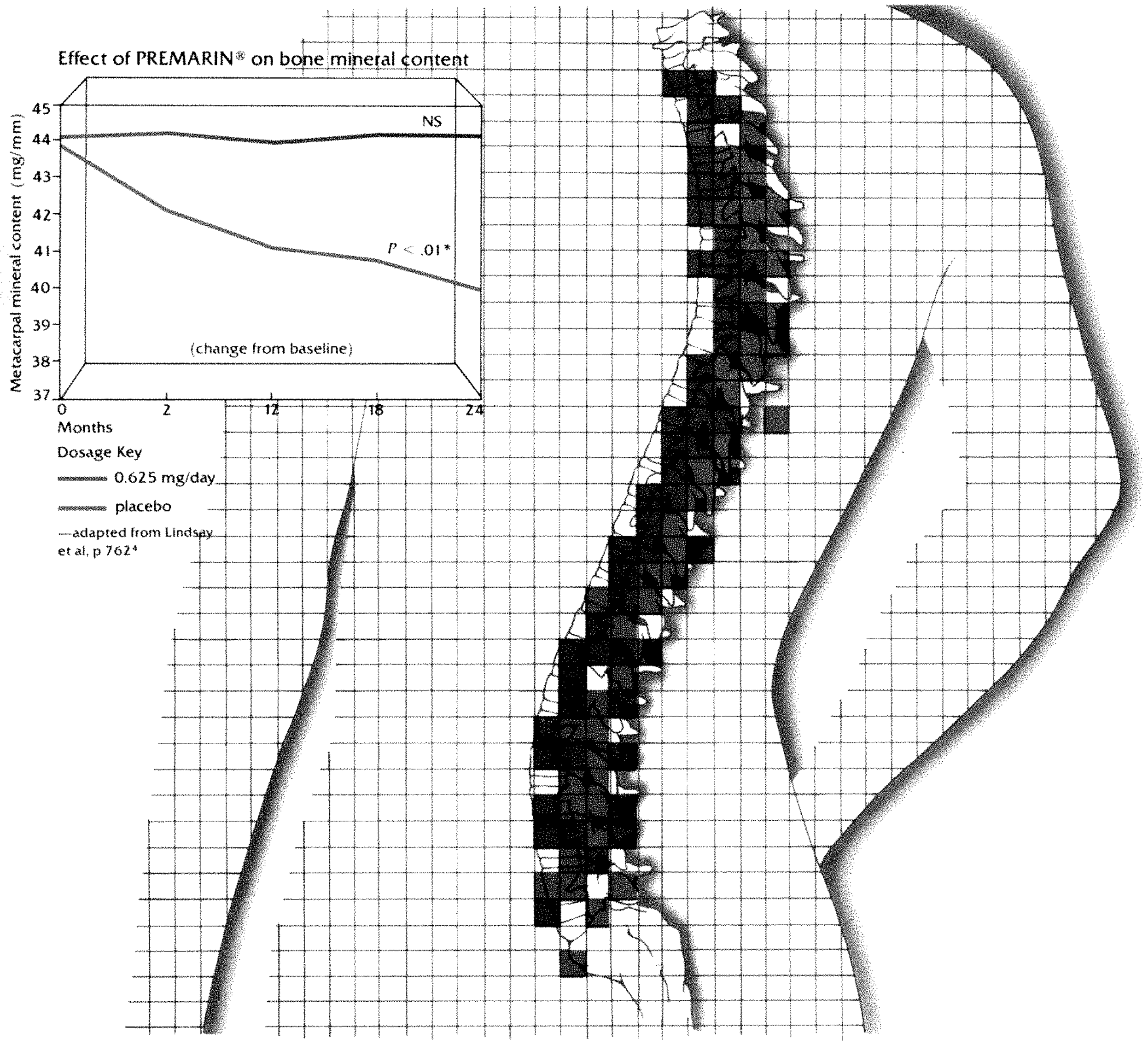
(CONJUGATED ESTROGENS, U.S.P.) Vaginal Cream



0.625 mg/g

Please see last page for brief summary of full prescribing information.

POSTMENOPAUSAL BONE LOSS THAT INCAPACITATES



Effect of PREMARIN® on bone mineral content

Dosage Key
- 0.625 mg/day
- placebo

—adapted from Lindsay et al, p 762[4]

PREMARIN MAY HALT THE DISABLING COURSE OF OSTEOPOROSIS*

Osteoporosis has an enormous epidemiological impact: it affects 10 million American women, and 26% of all women over age 60.[5] The disease begins silently and progresses inexorably for 15 to 20 years, until disabling complications occur.[6]

To minimize osteoporotic damage, the condition must be detected early and treated promptly. For many patients, PREMARIN is optimal therapy for osteoporosis, as part of a comprehensive program that includes exercise, good nutrition, and calcium supplements. In a controlled study of postmenopausal and oophorectomized women, PREMARIN (Conjugated Estrogens Tablets, U.S.P.) doses of 0.625 mg/day prevented loss of metacarpal mineral content (see graph above).

PREMARIN®

(CONJUGATED ESTROGENS TABLETS, U.S.P.)

0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

*Conjugated Estrogens Tablets have been evaluated as probably effective for treating estrogen-deficiency-induced osteoporosis.

Please see last page for brief summary of full prescribing information.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR)*

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.
PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P.) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences – National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)

2. Atrophic vaginitis
3. Kraurosis vulvae
4. Female hypogonadism
5. Female castration
6. Primary ovarian failure
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma – palliative therapy of advanced disease.
9. Postpartum breast engorgement – Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent study has raised this possibility. There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of th thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain an tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported i women taking estrogen-containing oral contraceptives. Increased blood pressure may occur wit use of estrogens in the menopause and blood pressure should be monitored with estrogen use. worsening of glucose tolerance has been observed in patients on estrogen-containing oral contracep tives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to sever hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be take prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estroge should not be prescribed for longer than one year without another physical examination bein performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardia or renal dysfunction, require careful observation. Certain patients may develop manifestations excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc Prolonged administration of unopposed estrogen therapy has been reported to increase the risk endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with a increased incidence of mental depression. Patients with a history of depression should be carefull observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The patholog should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develop in any patient receiving estrogen, the medication should be discontinued while the cause investigated. Estrogens should be used with care in patients with impaired liver function, rena insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in who bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increase norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormon as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decrease reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only whe clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including or contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhe premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterin fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretio cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdom nal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hai hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migrain dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydra tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN* Brand of Conjugated Estrogens Tablets, U.S.P.

1. *Given cyclically for short-term use only.* For treatment of moderate to severe *vasomotor* symptoms atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily

The lowest dose that will control symptoms should be chosen and medication should b discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. *Given cyclically:* Female hypogonadism. Female castration. Primary ovarian failure. Osteopor sis.

Female hypogonadism — 2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest perio of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule repeated. The number of courses of estrogen therapy necessary to produce bleeding may va depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cycli regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided dose for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occur before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day bleeding.

Female castration and primary ovarian failure — 1.25 mg daily, cyclically. Adjust upward downward according to response of the patient. For maintenance, adjust dosage to lowest level tha will provide effective control.

Osteoporosis (to retard progression) — 1.25 mg daily, cyclically.

3. *Given for a few days:* Prevention of postpartum breast engorgement — 3.75 mg every four hour for five doses, or 1.25 mg every four hours for five days.

4. *Given chronically:* Inoperable progressing prostatic cancer — 1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopaus women — 10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropria measures taken to rule out malignancy in the event of persistent or recurring abnormal vagin bleeding.

PREMARIN* Brand of Conjugated Estrogens, U.S.P. Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae:

The lowest dose that will control symptoms should be chosen and medication should b discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

Usual dosage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of th condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cance and appropriate diagnostic measures should be taken to rule out malignancy in the event of persiste or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865 — Each *purple* table contains 2.5 mg in bottles of 100 and 1,000. No. 866 — Each *yellow* tablet contains 1.25 mg in bottles 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864 — Each *white* table contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867 — Each *maroon* tablet contain 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and unit dose package of 100. No. 868 — Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is trademark of Ayerst Laboratories.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream – No. 872 — Each gram contain 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white wax glyceryl monostearate, propylene glycol monostearate, methyl stearate, phenylethyl alcohol, sodiu lauryl sulfate, glycerin, and mineral oil.)

Combination package: Each contains Net Wt. 1½ oz. (42.5 g) tube with one calibrated plasti applicator.

Also Available — Refill package: Each contains Net Wt. 1½ oz. (42.5 g) tube. **4340/285**

References: 1. Judd HL: After the menopause. *Transition* 1983;1:19-30. **2.** Erlik Y, Tataryn IV, Meldrum DR, et al: Association of waking episodes with menopausal hot flushes. *JAMA* 1981;245:1741-1744. **3.** Meldrum DR: The pathophysiology of postmenopausal symptoms. *Sem Reprod Endocrinol* 1983;1(February):11-17. **4.** Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **5.** Katz WA: *Rheumatic Diseases: Diagnosis and Management.* Philadelphia, JB Lippincott Co, 1977, p 672. **6.** Reese WD: A better way to screen for osteoporosis. *Contemp Ob/Gyn* 1983;22(November):116-131.

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Mycelex®-G: The original 3-night therapy for vulvovaginal candidiasis

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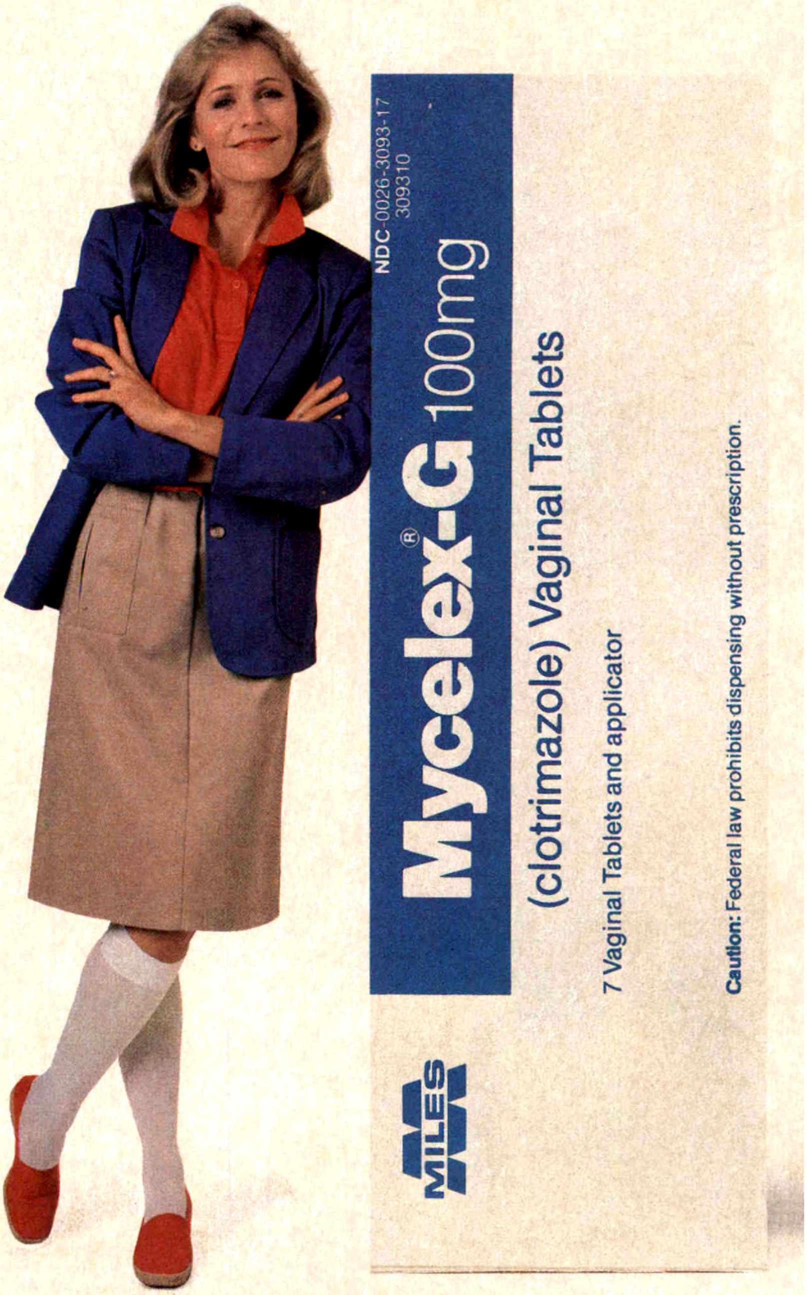
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309310
Mycelex®-G 100mg
(clotrimazole) Vaginal Tablets
7 Vaginal Tablets and applicator
Caution: Federal law prohibits dispensing without prescription.
MILES

Mycelex®-G

(clotrimazole) 100 mg vaginal tablets

MILES

Miles Pharmaceuticals

Reference:
1. Data on file, Miles Pharamaceuticals.

*Absence of signs and symptoms.

†Negative cultures.

‡For pregnant patients, the 7-day course of therapy must be used.

Please see next page for Brief Summary of prescribing information.

Miles Pharmaceuticals
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CP24,285

Patient accepted, non-waxy, 3-night therapy

Mycelex®-G

(clotrimazole) 100 mg vaginal tablets

ACTIONS: Clotrimazole is a broad-spectrum antifungal agent that inhibits the growth of pathogenic yeasts. Clotrimazole exhibits fungicidal activity *in vitro* against *Candida albicans* and other species of the genus *Candida*.

No single-step or multiple-step resistance to clotrimazole has developed during successive passages of *Candida albicans*.

INDICATIONS: Mycelex-G Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis (*Trichomonas* and *Hemophilus vaginalis)* should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex-G Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

PRECAUTIONS: Laboratory Tests: If there is a lack of response to Mycelex-G Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

Application of ^{14}C-labeled clotrimazole has shown negligible absorption (peak of 0.03 mcg/ml of serum 24 hours after insertion of a 100 mg tablet) from both normal and inflamed human vaginal mucosa.

Usage in Pregnancy: While Mycelex-G Vaginal Tablets have not been studied in the first trimester of pregnancy, use in the second and third trimesters has not been associated with ill effects. Follow-up reports now available on 71 neonates of 117 pregnant patients reveal no adverse effects or complications attributable to Mycelex-G therapy.

ADVERSE REACTIONS: Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints, such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet a day for seven consecutive days. Recent studies indicate that an alternative regimen of two tablets a day for three consecutive days is similarly effective in nonpregnant patients; however, in pregnant patients the three-day treatment course did not prove to be effective and is therefore not recommended. They should receive the seven-day treatment. Using the applicator supplied, insert one or two tablets intravaginally preferably at bedtime.

In the event of treatment failure, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy. There are no studies to show whether a second course of clotrimazole would be effective in patients who fail to respond to the initial course.

HOW SUPPLIED: Mycelex-G Vaginal Tablets 100 mg, white, tear-drop-shaped, uncoated tablets, supplied in a strip of seven tablets with plastic applicator and patient leaflet of instructions.

Do not store above 35°C (95°F).

DPSC Stocked: NSN 6505-01-090-6795

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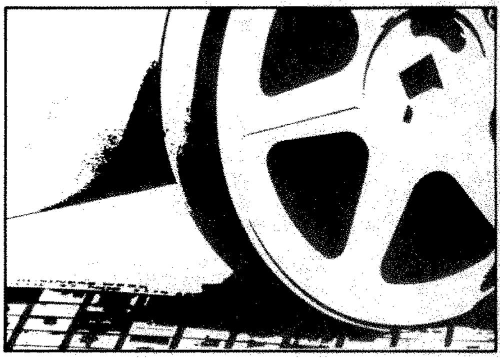

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The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England).

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

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General requirements for preparation of manuscripts

The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; departmental, divisional, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name. Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Number references consecutively in the order in which they are mentioned in the text. Use the format of the U.S. National Library of Medicine in *Cumulated Index Medicus.* Journal titles should also conform to abbreviations used in *Cumulated Index Medicus.*

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and 2 sets of unmounted prints on glossy paper. For black and white illustrations, submit 3 sets of 5 by 7 inch (8 by 10 inch maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name, title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). An average table or figure published in the JOURNAL is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures or used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints. Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Checklist

—Letter of submission
—Copyright transfer letter
—Original and two xerographic copies of manuscript
—Title page
  —Title of article
  —Full name(s) and affiliations of author(s)
  —Author to whom correspondence is to be sent
  —Reprint request line or line stating reprints not available
  —Short title
—Abstract (double spaced), 3 to 5 key words/phrases
—Article proper (double spaced)
—References (double spaced), on a separate sheet
—Legends (double spaced), on a separate sheet
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References:
1. Rössner S et al: *Acta Obstet Gynecol Scand* 59:255, 1980. 2. Nash AL et al: *Med J Aust* 2:277, 1979. 3. Larsson-Cohn U et al: *Fertil Steril* 35:172, 1981. 4. Briggs MH: *J Reprod Med* 28:92, 1983. 5. Åhrén T et al: *Contraception* 24:451, 1981. 6. Briggs MH, Briggs M: *Acta Obstet Gynecol Scand* Suppl 105:25, 1982.

***Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.**

See important information on reverse page.

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Indications and Usage—NORDETTE* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NORDETTE* each tablet contains
0.15 mg levonorgestrel and
0.03 mg ethinyl estradiol.
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EDITORIAL

Summary of analysis of published articles

September 1, 1983, through August 31, 1984

Each year a retrospective analysis of the articles published in the JOURNAL is made and is presented to the Editorial Board and to the Advisory Committee on Policy. During the past fifteen years we have compiled data on the classification and type of articles accepted and published. A comparison of the data for the last two years is presented. Readers and authors should note the time from acceptance to publication was 5.60 months for the period analyzed in 1984.*

*Publication time of articles for regular sections from article acceptance: 1983, 4.95 months; 1984, 5.60 months.

	1983			1984		
	No. of articles	Pages	Average length per article	No. of articles	Pages	Average length per article
Classification of article:						
Obstetrics	218	969		226	908	
Gynecology	190	948		220	975	
Fetus, Placenta, and Newborn	161	757		185	758	
Education	3	12		5	29	
Communication in Brief		192			207	
Total	572	2878		636	2877	
Type of article:						
Clinical	365	1542		302	975	
Clinical Investigation	98	503		231	1162	
Basic Research	109	641		103	533	
Communication in Brief		192			207	
Total	572	2878		636	2877	
Analysis by JOURNAL section:						
Regular: Obstetrics, Gynecology, and Fetus, Placenta, Newborn	301	1696	5.6	306	1554	5.1
Society	127	794	6.2	163	979	6.0
Communication in Brief	122	193	1.6	150	207	1.4
Current Development	9	107	11.9	9	97	10.8
Current Investigation				2	9	4.5
Clinical Opinion	13	88	6.8	6	31	5.2
Letter to the Editors		105			122	

Clinical Opinion

Spermicidal contraceptives and poor reproductive outcomes: The epidemiologic evidence against an association

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The first widely publicized report of an association between spermicidal contraception and congenital malformations and spontaneous abortion had considerable impact on obstetric practice. A large number of more recent epidemiologic studies have generally failed to support the earlier finding, and it is concluded that no such association has been demonstrated. The available evidence precludes the need for additional regulation of spermicidal contraception. (AM J OBSTET GYNECOL 1985;151:552-6.)

Key words: Congenital malformation, miscarriage, low birth weight, contraception, spermicide

The Maternal Health and Drugs Advisory Committee of the Food and Drug Administration was recently asked to address the following question. "Is there sufficient evidence in any of the present studies to warrant a special warning in the labeling concerning the use of vaginal spermicides during pregnancy?"[1] After a day of testimony the committee concluded that there was not sufficient evidence to warrant labeling spermicides, a decision which anyone familiar with the existing epidemiologic evidence would be hard pressed to find fault with.

Few practicing obstetricians will have been able to keep up with the rapidly growing literature on this topic, and an update of the current epidemiologic evidence is perhaps timely. In addition, epidemiologic studies are increasingly being used to provide evidence for or against regulations that directly influence obstetric practice, thus we present some observations on how epidemiologic inquiry relates to regulatory policy.

The 14 studies reviewed here represent all the known epidemiologic studies that have considered an association of spermicide use with congenital malformations, spontaneous abortion, or low birth weight.

The extant epidemiologic literature

Studies of congenital malformation. Congenital malformations have been the most frequently studied outcome associated with spermicides, with 10 studies reported in the literature. Table I (top section) lists these studies, shows their major design features, and reports their most important measures of association with a 95% confidence interval. Five case-control studies have been reported,[2-6] four of which found no association with spermicides, and there have been five prospective studies,[7-11] four reporting no association. Some studies examined selected congenital diagnoses whereas others reported for all malformations. All of the latter studies, however, failed to show an association of spermicide use with malformations, both when considered as a whole and when specific diagnostic subgroups were considered. Except where noted, the estimates reported in the table are unadjusted, since in none of the studies did adjustment for confounding variables materially change the estimate.

The one case-control study reporting a positive association[3] had a small number of cases (n = 16); although all infants had Down's syndrome, they were selected from a case series of patients with congenital heart defects and therefore represent a select group of newborn infants with Down's syndrome. Use of spermicides was ascertained simply as "during the period before this pregnancy" in determining the time of exposure. In contrast, the three most recent case-control studies[4-6] reported on larger numbers of newborn infants with chromosome anomalies (a total of 248 chro-

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Table I. Existing studies of spermicides and reproductive anomalies

Authors	*Year of publication*	*Type of study*	*No. of cases studied*	*Exposure measure*	*Exposure closest to conception*	*Measure of association*		*Comment*
						*Estimate**	*95% confidence limit†*	
Congenital anomalies								
Smith et al.[2]	1977	CC	93	SR	"Method of contraception"	3.0	0.9, 9.7	Limb defects
Rothman[3]	1982	CC	16	SR	"In period before pregnancy"	3.5‡	1.1, 11.0	Down's syndrome with heart disease
Polednack et al.[4]	1982	CC	715	SR	After last menstrual period	0.7 2.0	0.2, 2.2 0.5, 7.8	Chromosome anomalies Limb defects
Codero and Layde[5]	1983	CC	188	SR	Periconceptual	1.2 1.0	0.4, 3.3 0.3, 3.3	Chromosome anomalies Limb defects
Bracken and Vita[5]	1983	CC	1427	SR	At conception	1.3	0.8, 1.8	All malformations
Jick et al.[7]	1981	Pr	56	P	≤600 days of delivery or spontaneous abortion	2.2‡	1.3, 3.9	All malformations
Huggins et al.[8]	1982	Pr	193	SR/P	Diaphragm as "main" method	1.7 1.7	0.3, 8.8 0.3, 8.8	Chromosome anomalies Limb defects
Mills et al.[9]	1982	Pr	1420	SR	After last menstrual period	1.0	0.9, 1.2	All malformations
Shapiro et al.[10]	1982	Pr	2277	SR/P	≤16 weeks of last menstrual period	1.1	0.7, 1.6	All malformations
Linn et al.[11]	1983	Pr	58	SR	"Last method used before pregnancy"	0.8	0.7, 1.1	All malformations (adjusted)
Spontaneous abortion								
Scholl et al.[12]	1983	CC	363	SR	At conception	2.7‡	1.3, 5.8	
Harlap et al.[13]	1980	Pr	1507	SR	After last menstrual period	1.1	NE	Age adjusted
Jick et al.[14]	1982	Pr	222	P	≤12 weeks of conception	1.8‡	1.3, 2.4	
Huggins et al.[8]	1982	Pr	671	SR/P	Diaphragm as "main" method	1.0 0.6‡	0.9, 1.2 0.5, 0.8	Planned pregnancies Unplanned pregnancies
Low birth weight								
Scholl et al.[12]	1983	CC	2528	SR	At conception	NE	NE	No significant decrease of birth weight with spermicides
Polednack et al.[4]	1982	Pr	1018	SR	After last menstrual period	0.6 1.3	0.1, 4.2 0.5, 3.6	Male newborn infants Female newborn infants

CC, Case-control; Pr, prospective; SR, self-report; P, prescription; NE, no estimate. The odds ratio is used for case-control and the relative risk for prospective studies.

*These were recalculated for some studies but not estimable for others.

†95% confidence limit calculated by means of the variance of the log of the estimate.

‡$p < 0.05$.

mosome anomaly cases in all three studies) and, as shown in Table I, obtained greater precision in determining when exposure occurred with respect to conception.

Among the five prospective studies the first report of Jick et al.[7] is the only one to report a positive association between spermicide use and congenital malformations. The study of Jick et al. can be criticized on at least four counts. First, the measure of spermicide use was extremely broad, i.e., a prescription filled within 600 days of delivery. It is most likely that many of the "exposed" subjects were not actually exposed around the time of conception. For example, in the Yale study[6] only 27% of the control subjects using spermicides as recently as 2 months before conception actually used them at conception itself. Moreover, in the study of Jick et al.[7] there is no certainty that the "unexposed" group of women had not used spermicides.[15] The second difficulty with this study is the low number of malformations observed. Not only were few cases studied (56 versus a range of 58 to 2277 for the other prospective studies), but the rate of malformation in the unexposed population was low (1.0%) compared with many studies of hospital origin which range from 2% to 5% depending upon definition. In the spermicide-exposed group in Jick et al.'s study the malformation rate was only 2.2%. Third, the asemblage of malformations Jick et al. identified as being associated with spermicides—Down's syndrome, limb reductions, hypospadias, and malignant neoplasms—all lack a common etiology, making it very unlikely that spermicides would be a shared risk factor. For example, trisomy 21, which causes Down's syndrome, occurs at conception whereas hypospadias may occur relatively late in the first trimester of pregnancy. Limb reduction defects are themselves of mixed etiology, occurring either as a failure in the development of the limb bud or because of positional intrauterine pressure resulting in necrosis of a previously developed normal limb. Fourth, it is not clear that Jick et al. adequately controlled their study for confounding variables. Only maternal age was adjusted, and nothing is reported about the control of other potentially confounding factors.

Because of the rarity of individual diagnoses of congenital malformation, many studies have low statistical power for identifying moderate risks caused by spermicides at statistically significant levels. It is important to note, therefore, that the case-control study of Bracken and Vita[6] and prospective study of Shapiro et al.[10] were large enough to detect relative risks of Down's syndrome of the size reported by Rothman[3] but failed to do so. Similarly, an increased risk of limb defects of the order reported by Jick et al.[7] could have been detected in the study of Mills et al.[9] but was not.

Miscarriage studies. Four prospective studies have examined the association of spermicidal contraceptives and risk for miscarriage (middle section of Table I). Jick et al.[14] first reported a positive association in their 1981 paper (reviewed above) and expanded their analysis in 1982 with use of the same population as that examined for congenital malformations.[14] While some methodologic ambiguities were improved in the later study, other problems persisted, the most important being a failure to adjust for any potentially confounding factors. This is particularly troublesome in the comparison of women using spermicides in the 12 weeks before conception. Probably many of these pregnancies are "unplanned" and therefore occur in women known to be of higher risk for spontaneous abortion. The comparison with women recently using oral contraceptives is at risk of being biased, since several studies have suggested that recent oral contraceptive use protects against miscarriage[13]; however, this itself remains a contentious issue. Huggins et al.[8] found no positive association of spontaneous abortion with pregnancy in a study that had a higher spontaneous abortion rate than in Jick et al.'s study (13.8% in the unexposed group compared with 3.3% in Jick et al.'s study) but had an imprecise measure of spermicidal exposure which was limited to being the "main birth control method in use at any time." In this study women who experienced an unplanned pregnancy and were using the diaphragm with spermicides close to conception had a significantly reduced risk of spontaneous abortion.

Scholl et al.[12] have recently analyzed the National Survey of Family Growth and reported an association of spermicide use with spontaneous abortion. This study also has several major difficulties including (1) comparing women exposed to spermicides close to conception with those exposed after conception which, as in the Jick et al. study, does not account for confounding caused by breakthrough pregnancies occurring in women who are likely to be at higher risk of spontaneous abortion than those who plan to stop contraception; (2) asking women about all their pregnancies over a prior period of almost 4 years, which enhances the chance of biased recall of spermicide use in women experiencing spontaneous abortion, and (3) using a fixed observation period so that women with a history of multiple spontaneous abortions are likely to be overrepresented in the sample. Women with multiple spontaneous abortions may be especially likely to use spermicides and have frequent pregnancies and so be counted more than once in the analysis. By far the better-designed and most convincing investigation is the large cohort study (n = 32,123) of Harlap et al.[13] which found no association between spermicide use and spontaneous abortion. Overall therefore, current evidence is against there being an association between spermicidal contraception and spontaneous abortion.

Low birth weight. Birth weight is the third reproductive outcome studied, although by only two research groups (lower section of Table 1). Polednack et al.[4] reported spermicide use after the last menstrual period was significantly associated with lower average birth weight in female offspring but only in the male offspring of mothers who smoked. Spermicide use before the last menstrual period exerted no effect on birth weight. It should be noted that all the variables studied in the analysis accounted for only 14% to 20% of the variation in birth weight. Additionally, the proportion of clinically low–birth weight infants was not significantly influenced by spermicide usage. Scholl et al.[12] found no association between spermicidal exposure and lower birth weight.

Epidemiologic evidence and public policy

The bias of first studies. It is probably no coincidence that the first, most widely publicized spermicide study was the positive one of Jick et al.[7] in 1981. It is quite common in epidemiology for the first published studies of an association to suggest a positive association, with subsequent reports being negative; a review of the oral contraception and malformation studies would provide another example. The reason for this is fairly obvious. Investigators are more likely to write up positive findings, reviewers to consider them of interest, editors to publish them, and the press to publicize them. It is only after the initial observation is published that investigators who have negative data feel obliged to report them. For example, we observed the absence of a spermicide association in the Yale data in 1977 but gave little priority to a full analysis and writing of the paper until after the Jick et al. paper made headlines in 1981. The initial reports of association are also often based on secondary analyses of existing data, and specifically designed studies come later. A number of large studies specifically designed to test the spermicide-malformation hypothesis are at present only in their data collection stage. Thus several epidemiologic studies should be in the literature before the literature can be reviewed as evidence for public policy.

Risk-benefit of product and regulatory policy. In considering whether to regulate a product, two risk-benefit analyses need to be considered: (1) the risk-benefit of the product itself and (2) the risk-benefit of regulation. For spermicidal contraceptives the benefit considerations are straightforward. Spermicides are an important, indeed the only, form of contraception available to many women, especially for those who for health reasons may not be able to use oral contraceptives or an intrauterine contraceptive device, for women who cannot rely on their partner to practice rhythm and withdrawal or use condoms, and for women who are advised to delay conception after discontinuing use of oral contraceptives or the intrauterine contraceptive device and for women not wishing to be sterilized. The potential risks of spermicides are the reproductive effects reviewed in this report.

The benefits and risks of regulation itself are perhaps less obvious. The benefits of regulation would include the avoidance of a number of congenital malformations and spontaneous abortions if the reported associations are in fact real. On the other hand, the risks of regulation, in light of the available weak evidence against spermicides, are considerable. They include devaluing both the regulatory process and the credibility of epidemiologic research in the public's view, particularly if subsequent research confirmed the lack of an association and regulations had to be reversed. Regulation of spermicides would also have a deleterious effect on individual behavior. It would almost certainly lead to an increase in unprotected intercourse with an increase in unplanned conceptions. It is also likely that women who conceived while using spermicides would be made extremely anxious about their pregnancies, some even to the extent of requesting induced abortions for pregnancies that might otherwise be wanted. Publication of the Jick et al. paper in 1981 led directly to requests for amniocentesis and ultrasound among patients of obstetricians practicing at Yale–New Haven Hospital.[16] Ironically, amniocentesis itself carries a small but documented risk (1% to 2%) of inducing spontaneous abortion.

The strength of epidemiologic evidence and regulatory policy. The degree of evidence necessary for regulation is a function of at least two considerations: (1) whether regulation is to identify a product as being safe, that is, free of risk (e.g., the use of a drug in pregnancy); or whether a product is being labeled as hazardous (e.g., oral contraceptive use in women over 35 years of age who smoke); and (2) the overall social risk-benefit of the product as just described.

For all products I believe we should demand stronger epidemiologic evidence of safety than we might require in suggesting a hazard. It is in the public's greater interest to label a product as hazardous than to sanctify it as safe. The second consideration is that of the product's overall social benefit. When a product has overall social benefit (i.e., greater social benefits than risks), regulatory policy should demand even stronger epidemiologic evidence before accepting an association of the product with a particular health risk.

Regarding the decision on whether to label spermicidal contraceptives as a potential hazard, the situation is one in which not only do the products have social benefits but the epidemiologic evidence for an association with poor reproductive outcomes is extraordinarily weak. A comparison may be made for products with no social benefit (thalidomide and cigarettes) but with strong evidence for their association with reproductive problems. In these examples regulatory policy in the form of banning thalidomide and labeling cigarettes is clearly justified. For spermicidal contraceptives, however, the present epidemiologic evidence is too weak to support additional regulation. Moreover, any such regulation would incur significant social risks and, on the basis of currently available evidence, be contrary to the public's best interest.

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The scheduling of repeat cesarean section operations: Prospective management protocol experience

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There are benefits to patients and a busy obstetric service if repeat cesarean section operations are performed on a scheduled basis. Optimum management avoids prematurity and reduces the need for amniocentesis. Over a period of 20 months repeat cesarean sections were performed at Tripler Army Medical Center while a protocol with the following elements was used: (1) known last menstrual period; (2) landmarks: positive urine human chorionic gonadotropin test by 6 weeks, Doppler fetal heart tone by 12 weeks, date determination by examination before 10 weeks, fetoscope fetal heart tone by 20 weeks, and date determination by size before 30 weeks; (3) date determination by midtrimester sonogram(s); (4) normal third-trimester glucose screening; (5) biparietal diameter of 9.2 or 9.5 cm before scheduling. With two or more clinical landmarks and one date by sonogram or one landmark and date by two sonograms, elective repeat cesarean section was scheduled at 39 weeks if the biparietal diameter was ≥ 9.2 cm (127). If dates by sonogram were less than dates by last menstrual period but greater than 1 week or if last menstrual period was unknown, dates by sonogram and landmarks corresponding to dates by sonogram were used to electively schedule, with biparietal diameters of 9.2 or 9.5 cm respectively required (28). If protocol criteria were not met or earlier delivery was indicated (e.g., vertical scar or diabetes), amniocentesis was performed (42), except when not possible, advisable, or refused when patients either elected labor (20) or were scheduled if three or more criteria for 40+ weeks were met (18). Of 225 patients (70.5%) scheduled by protocol (173), amniocentesis (34), or medical indication (18), 188 (58.9%) were delivered without labor. In the 147 patients (46.1%) delivered electively by protocol without labor or amniocentesis, there were no cases of respiratory distress syndrome and the mean birth weight was 3517 gm. With early care and better patient compliance nearly all repeat cesarean sections can be safely delivered electively with the use of this protocol. (AM J OBSTET GYNECOL 1985;151:557-63.)

Key words: Repeat cesarean section

Much has been written lately promoting the idea of vaginal delivery after cesarean section operations. The fact remains that for many patients, and in many delivery circumstances, repeat cesarean section remains the choice. There are benefits to both patients and to a busy obstetric service if repeat cesarean sections are performed on a scheduled elective basis, provided the risks of prematurity and respiratory distress syndrome[1, 2, 3] can be avoided.

Some authors[4, 5] would suggest the routine use of amniocentesis for the assessment of fetal pulmonary maturity with amniotic fluid studies before any elective delivery. However, it must be remembered that there are certain hazards with the performance of amniocentesis.[6, 7] Also in many cases, because of placental position or other factors, amniocentesis cannot easily be performed.

Another alternative would be to allow repeat cesarean patients to enter labor first.[3] While the risk of serious consequences of labor with a previous low transverse scar is very small,[8-12] this approach does not offer reassurance when the previous scar is vertical or unknown, and it negates the advantages of scheduling.

An optimum management approach would eliminate prematurity and at the same time greatly reduce the need for amniocentesis or labor before a repeat cesar-

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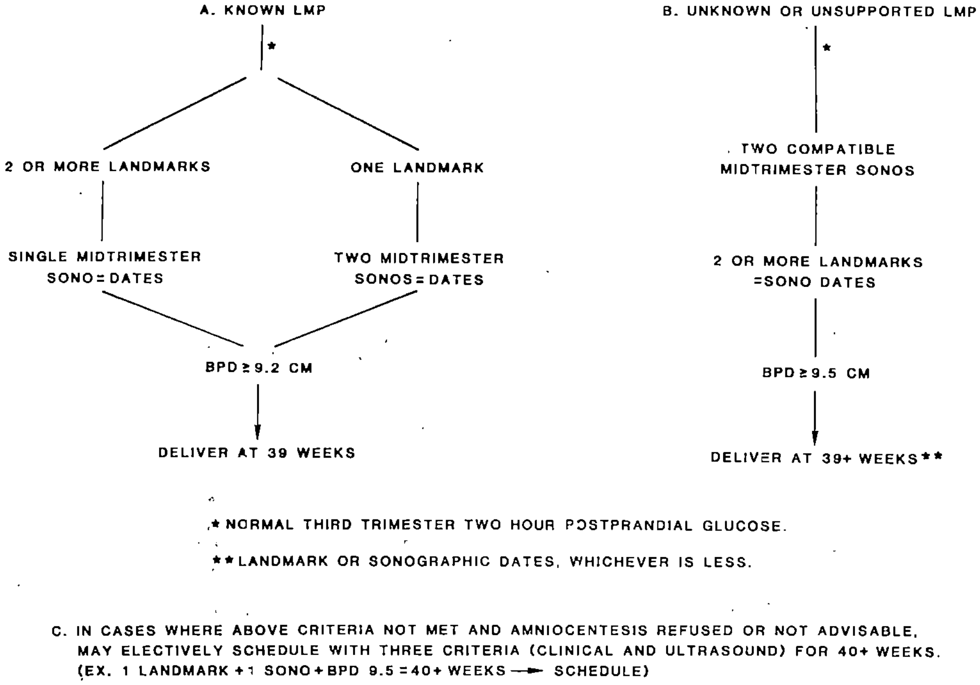
A. KNOWN LMP
*
2 OR MORE LANDMARKS
ONE LANDMARK
SINGLE MIDTRIMESTER SONO = DATES
TWO MIDTRIMESTER SONOS = DATES
BPD ≥ 9.2 CM
DELIVER AT 39 WEEKS
B. UNKNOWN OR UNSUPPORTED LMP
*
TWO COMPATIBLE MIDTRIMESTER SONOS
2 OR MORE LANDMARKS =SONO DATES
BPD ≥ 9.5 CM
DELIVER AT 39+ WEEKS**
* NORMAL THIRD TRIMESTER TWO HOUR POSTPRANDIAL GLUCOSE.
** LANDMARK OR SONOGRAPHIC DATES, WHICHEVER IS LESS.
C. IN CASES WHERE ABOVE CRITERIA NOT MET AND AMNIOCENTESIS REFUSED OR NOT ADVISABLE, MAY ELECTIVELY SCHEDULE WITH THREE CRITERIA (CLINICAL AND ULTRASOUND) FOR 40+ WEEKS. (EX. 1 LANDMARK + 1 SONO + BPD 9.5 = 40+ WEEKS → SCHEDULE)

Fig. 1. Criteria for elective repeat cesarean section without amniocentesis. *LMP,* Last menstrual period; *BPD,* biparietal diameter.

ean section is performed. Toward this end, clinical parameters for estimating gestational age and avoiding preterm delivery have been reassessed[13] and protocols combining clinical parameters and midtrimester ultrasound have been employed,[14, 15] with little or no incidence of respiratory distress syndrome.

The increased availability of obstetric ultrasound has suggested its use as a final scheduling parameter for elective repeat cesarean sections. Lee et al.[16] found no infants of <2500 gm with a biparietal diameter >8.7 cm, and Hayaski et al.[17] found no cases of hyaline membrane disease in which the biparietal diameter was ≥9.3 cm after 38 weeks' gestation. However, Goldstein et al.[18] found a 24% incidence of lethicin/sphingomyelin ratios <2 when a minimum biparietal diameter of 9.0 cm alone was used; although Golde et al.[19] had no cases of respiratory distress syndrome, a 7% incidence of lethicin/sphingomyelin ratios <2 were found when a minimum biparietal diameter of 9.2 cm alone was employed.

Prior experience at Tripler Army Medical Center had indicated that a combination of good dating parameters, including midtrimester ultrasound confirming dates and a biparietal diameter of ≥9.2 cm after 38 weeks' gestation could in the absence of diabetes mellitus virtually guarantee the absence of hyaline membrane disease in the newborn infant. The aim of the present study was to evaluate the performance of such a management protocol as applied to the elective scheduling of repeat cesarean sections.

The management protocol

All patients attending the obstetric clinic at Tripler Army Medical Center who had undergone one or more previous cesarean sections were evaluated according to the scheme shown in Fig. 1. The patient's last menstrual period was determined if possible, as well as its normalcy, regularity, and interval and whether or not oral contraceptives were used. Information regarding the number, type, and indications for the prior cesarean section(s) was also placed in the obstetric record at the first visit or requested from the previous physician. Because of possible delay in respiratory maturation in diabetic pregnancies, a diabetic screen consisting of a fasting and 2-hour postprandial glucose was ordered during the second and third trimesters (32 to 36 weeks).

A prospective attempt was made to obtain as many as possible of the following clinical landmarks: (1) Positive urine pregnancy test by the sixth week of gestation. (2) Positive Doppler fetal heart tone by the 12th week gestation. (3) Early dates corresponding to examination before the tenth week of gestation. (4) Fetoscope fetal heart tone by the twentieth week of gestation. (5) Dates corresponding to uterine size before 30 weeks of gestation.

During this period all patients who reported early enough for obstetric care received a screening ultrasound examination (performed in the obstetric clinic usually at 18 to 24 weeks' gestation) by means of a real-time scanner with a 3.0 or 3.5 mHz focused transducer (ADR, Inc., Tempe, Arizona). Biparietal diameters were obtained by means of the internal electronic caliper to measure the largest outer-to-inner table dimension with clear symmetrical midline anatomy at the level of the thalamus. Gestational age was estimated by means of the table from Sabbagha et al.[20] Whenever possible, a second ultrasound scan was also obtained during the second trimester.

At ≥38 weeks' gestation the patient was evaluated for scheduling. At this time clinical dating landmarks and ultrasound scans were reviewed and a final ultrasound examination was performed. In those patients with a known last menstrual period, negative glucose screen, two or more landmarks with at least one midtrimester sonogram that determined dates within −1 week and +2 weeks, or one landmark with at least two midtrimester sonograms determining dates, elective repeat cesarean section was scheduled at ≥39 weeks' gestation depending on schedule availability, if the biparietal diameter was ≥9.2 cm. In cases where the last menstrual period was unknown or uncertain, the combination of two landmarks which equaled the dates as determined by two or more sonograms and a biparietal diameter of 9.5 cm also allowed scheduling at 39+ weeks' gestation. All cases which failed to qualify for elective scheduling by the above criteria were to have amniotic fluid pulmonary maturity studies if elective delivery prior to the onset of labor was planned. In cases where amniocentesis was either not possible or inadvisable because of placental position or lack of a safe fluid pocket ("tap site") or where amniocentesis was refused, the patient was scheduled to be allowed to enter labor. However, if in waiting for labor the patient reached the point where three or more criteria (clinical and ultrasound) were present which indicated 40+ weeks' gestation, she could then be electively scheduled.

In those cases with a previous vertical scar, amniocentesis, and delivery at 38 weeks' gestation, if possible, was planned. Amniocentesis prior to delivery was also to be employed in all cases of diabetes mellitus. Delivery without either amniocentesis or satisfaction of protocol requirements was allowed for either maternal (e.g., preeclampsia) or fetal (e.g., definite intrauterine growth retardation or distress) indications. In cases of premature labor or rupture of membranes, the cesarean section was repeated generally if the patient was beyond 36 weeks' gestation. If the gestation was felt to be possibly <36 weeks, tocolysis and assessment of pulmonary maturity and possible infection was undertaken, if possible, before appropriate therapy, delay, and repeat cesarean section was performed.

All maternal and neonate charts were reviewed by the author. Respiratory distress syndrome was determined by the typical clinical and x-ray assessment, combined with the requirement for supplemental oxygen, and confirmed by the neonatologist.

Table I. Method of scheduling and results for scheduled patients

Group	*Scheduled*	*Delivered electively*	*Labor before scheduled date*
Protocol A	127 (39.8%)	114 (35.7%)	13
Protocol B	28	24	4
Protocol C	18	9	9
Total protocol groups	173 (54.2%)	147 (46%)	26 (8.2%)
With amniocentesis	34 (13.2%)	26	8
With maternal or fetal indications	18 (5.6%)	15	3
Total patients	225 (70.5%)	188 (58.9%)	37 (11.6%)

Results

During the 20-month period from October 1, 1980, to May 31, 1982, 332 patients with previous cesarean sections were delivered at Tripler Army Medical Center. Repeat cesarean sections were performed on 319 (96%) of these patients. There were nine planned vaginal attempts, with eight being successful, and an additional five unplanned vaginal deliveries of patients who presented in advanced labor prior to scheduling (at 35, 37, 37, 37, and 38 weeks). Included in the study population were 71 patients who had more than one previous cesarean section, including 10 who had three previous cesarean deliveries. Nine patients were known to have had a previous vertical incision, three of which were classical in type. There were 158 (47.6%) cases in which the uterine scar was undocumented or unknown. In all of these cases the uterine scar was suspected to be low transverse in type and indeed this proved to be the case at operation.

Among the repeat cesarean section patients the last menstrual period was known in 299 cases (93.7%), although only 233 (73%) patients were able to state that they were both sure of the date and that their periods were regular. In the study population prenatal care and evaluation were obtained in sufficient time so that 85.9% had at least one clinical landmark for the given last menstrual period and 93.3% were able to have at least one ultrasound scan before 29 weeks' gestation. The stated last menstrual period was confirmed within ±7 days on the initial second-trimester scan in 178 patients. However, on the initial scan there were 39 cases in which the biparietal diameter estimation of gestational age differed by >14 days plus or minus from estimation by the last menstrual period, including 13 cases in which the date had been stated to be sure *and* the period to be regular. A second midtrimester scan was obtainable in 67.5% of cases.

Glucose screening revealed 11 cases of diabetes mellitus (3.4%), with two requiring insulin (nine Class A and two Class B).

The patients undergoing repeat cesarean sections

Table II. Patients with previous cesarean section who entered labor (total population = 332)

	No. of patients	*Week of gestation*	%
Elected labor	20	39-42	6.0
Labor before scheduled date:			
Protocol	29	>38 (all)	8.7
Amniocentesis	16	<38 (8)	4.8
Labor before scheduled			
Cesarean section	65	<38 (27) 38-39 (24) 39 (15)	19.9
Vaginal delivery	5	35,37,37,37,38	1.5
Planned and delivered vaginally	8	>38 (all)	
Total No. of patients	144		43.4%

who were able to be scheduled, the method of scheduling, and resultant type of delivery, whether elective or in labor, are presented in Table I. Of the patients scheduled from the protocol group A, 84% had two or more clinical landmarks. In these patients the percentage of clinical landmarks present were as follows: date determination by size (99+%), fetoscope fetal heart tone by 20 weeks (75%), dates determined by examination before 10 weeks (23.5%), Doptone fetal heart tone by 12 weeks (9%), and positive urine pregnancy test by 6 weeks (10%). The average gestational age at delivery in group A ($n = 114$) was 275.6 days (39.5 weeks). Reasons for scheduling in the group of those delivered for maternal or fetal indications included good estimated dates with suspected intrauterine growth retardation (11), oligohydramnios (2), worsening chronic hypertension or preeclampsia (4), and anti-c,E sensitization with no tap site. Five of these patients were delivered between 37 to 38 weeks' gestation.

A total of 42 amniocentesis procedures were performed, with eight at the time of premature labor. There were 16 cases in which the biparietal diameter was ≥ 9.2 cm. In 14 of these, the lethicin/sphingomyelin ratio was ≥ 2.0, but in two cases, both with biparietal diameter equaling 9.6 cm and *no* clinical landmarks, the lethicin/sphingomyelin ratios were 1.8 and 1.9. Delivery was delayed a week in both cases, and neither infant developed respiratory distress syndrome. There was one patient for whom amniocentesis failed to obtain fluid. She then elected to labor before having repeat cesarean section.

All patients with previous cesarean sections who entered labor prior to repeat cesarean section or vaginal delivery are characterized in Table II. In all there were 40/332 patients (12%) who entered labor and were delivered prior to 38 weeks' gestation. An additional five patients were delivered because of indications before 38 weeks. The preterm (38 week) rate is then 13.6%, with 21 of 332 potential and 20 of 319 repeated cesarean sections (both 6.3%) delivered before completing 37 weeks. There were another 33 of 332 patients (9.9%) delivered between 266 and 272 days from the last menstrual period, leaving only 255 of 332 patients (76.8%) possibly available for scheduling at 39 weeks (273 days) or greater. Because of the lack of schedule space and operating time, patients were often scheduled 3 to 4 days later than they could have been, with the result that 19 scheduled patients labored after 39 weeks' gestation and before their scheduled date. At least two patients in the group electing labor could have been scheduled on further observation, while on the other hand 22 patients who initially did not meet criteria on first examination after 38 weeks (usually biparietal diameter of <9.2 cm) were able to be scheduled with an additional period of observation (usually 1 week).

At the time of delivery, general anesthesia was used in 29.3% of electively delivered patients and 51.1% of those in labor. Except for two epidural injections, spinal anesthesia was the predominent anesthesia for delivery, having been utilized in 61% (195) of all patients. Of the 157 sterilization procedures performed (49.2%), six were cesarean hysterectomies: five elective for gynecologic indications and one an emergency procedure for uncontrollable hemorrhage and uterine atony.

There were no maternal mortalities. The average hospital stay after delivery was 3.9 days in the laboring patients and 3.8 days in those without labor (excluding one patient with peripartal cardiomyopathy and a 26-day postpartum stay). The usual stay for the overwhelming majority of patients (87.5%) was 3 or 4 days. Only 15 patients (4.7%) stayed >5 days. The estimated blood loss exceeded 1000 ml in only 16 cases (5%). Twelve patients (3.8%) received blood transfusions, five in the labor group and seven in the nonlabor group. Transfusions were used in three cases of placenta previa, one case with von Willebrand's disease, a uterine artery laceration, and the emergency hysterectomy. None of the elective cesarean hysterectomies required transfusions.

During the period under study, cefamandole nafate irrigation[21] of the operative site was used routinely in all nonallergic patients. There was only one case of endometritis in the electively deliverd nonamniocentesis patients (1 of 162, or 0.6%). This is a significantly different rate than in laboring patients: 6 of 131, or 4.6% ($p < 0.05\ \chi^2$). There were three cases (11.5%) in the 26 performed electively after amniocentesis ($p < 0.05$ compared to elective nonamniocentesis deliveries) for an overall rate of 10 of 319, or 3.1%. The rate of urinary tract infections was also significantly greater ($p < 0.01$) in the laboring group (13 of 131, or 9.9%) than in the nonlaboring patients (4 of 188, or 2.1%).

The incidence of scar dehiscence, or "window," discovered at the time of cesarean section was 3 of 131, or 2.3%, in the labor group and 7 of 188, or 3.7%, in the nonlaboring patients. One case occurred in the 13 vaginal deliveries for an overall incidence of 3.3% (11 of 332). None of these posed a serious problem, and all at the time of cesarean section were repaired with the uterine closure without difficulty.

There was a single antepartum fetal death in this series which was allowed to be delivered vaginally at 38 weeks and a single neonatal death (a 4120 gm female infant delivered after mature amniocentesis, with a thoracolumbar open neural tube defect, Arnold-Chiari malformation, and gut malrotation, of sepsis at 20 days of age). There were no instances of respiratory distress syndrome among any of the electively delivered infants. However, three cases occurred in infants delivered at 33+ weeks (1880 gm), 36+ weeks (3430 gm), and 37 weeks (2998 gm). These infants were hospitalized for 19, 10, and 16 days, respectively. In all, only nine infants remained hospitalized after the mother's discharge: the above three plus an infant with intrauterine growth retardation, a poorly feeding premature infant in the labor group (5), a small–for–gestational age infant following meconium aspiration, a large–for–gestational age (5470 gm) infant after amniotic fluid aspiration with persistant fetal circulation, one hyperbilirubinemic term infant, and the infant who later died.

The mean birth weights of the electively delivered groups were protocol A group, 3500 ± 470 gm; protocol B group, 3555 ± 377 gm; and protocol C group, 3650 ± 533 gm. These compare with a mean birth weight of 3374 ± 429 gm for the group with elected labor and 3375 ± 394 gm for the group with amniocentesis but not in labor. The electively delivered vaginal group averaged 3638 ± 348 gm. In the 114 patients delivered electively from protocol A, there were six infants <2900 gm, all of whom were estimated to be of 40 weeks' gestation on neonatal examination (two small–for–gestational age infants.) In this same group there were 15 infants >4000 gm.

Comment

The decision to routinely perform a repeat cesarean section has recently been questioned[22] in view of suggested disadvantages to patients in terms of cost, avoidance of iatrogenic prematurity, and maternal morbidity. Also, as previously noted, a trial of labor appears safe and has a reasonably good chance of success in the properly selected patient. There are, however, safety requirements[8] that are not always available (e.g., immediate operating room availability and 24-hour on-floor anesthesia) to every obstetric service. Furthermore, although many patients are interested in a vaginal delivery attempt, many elect to undergo repeat cesarean section either initially or as term approaches. In addition, others are removed because of risks as the pregnancy progresses.

The advantages of elective scheduling for the patient relate to family and job considerations but also to decreased possibility of aspiration pneumonia, a greater likelihood of conduction anesthesia, and decreased potential infectious morbidity when labor and ruptured membranes prior to cesarean section are avoided. Advantages to the busy obstetric, anesthesia, and neonatal services in terms of planned staffing and availability of back-up assistance are obvious.

Of the potential disadvantages to the patient, there is no real difference in cost to the military patient between vaginal and cesarean delivery. Despite infection rates in this series as low or lower than those usually ascribed to vaginal delivery,[23] there was still a significantly increased risk of infection for the laboring patient. In most situations the additional hospital days for the infected patient can be quite costly. Although the overall maternal morbidity was extremely low in this series, one might suspect that it still would be more than with vaginal delivery. However, most of the major problems or complications in these patients (e.g., blood transfusion) were the result of medical or obstetric circumstances that did not directly relate to the cesarean section operation.

The ability to electively schedule repeat cesarean sections without the need for amniocentesis and without iatrogenic prematurity and respiratory distress syndrome was the major thrust of this management protocol. This relatively conservative management scheme sought to combine ideas and lessons previously recorded or suggested in the literature with past experience at this institution.

Although a certain and reliable last menstrual period is the most reliable indicator of gestational age,[24] Hertz et al.[13] found that to be 90% sure of its being 38 weeks, it needed to be 42 weeks by last menstrual period. The number of patients (12.2%) in this series for whom initial dates by sonogram differed from dates by the last menstrual period by more than two weeks attests to the hazards of total reliance upon the last menstrual period even if it is "certain." Although Andersen et al.[25] states that in patients with a known last menstrual period additional clinical information did not improve the prediction of a delivery date, one suspects that the information is valuable in differentiating the true "certainty" of the period. In addition, in a highly mobile, multiracial, and military population such as ours, multiple additional factors may affect the "certainty" of the stated last menstrual period as opposed to that of a stable private population.

The standard error in the prediction of the delivery date observed by the above authors may as much reflect the biologic variation of "term" and the bias as to when the clinical landmarks were obtained as any error in their inherent predictiveness. The observations that an estimated date of confinement predicted by several clinical examinations and landmarks can be as accurate as a known last menstrual period,[25] even when differing from a given last menstrual period[26] provides support for the various aspects of the management scheme.

The use of one or more ultrasound examinations during a period when a 90% confidence interval is ± 10 days[20] is a powerful tool for the confirmation of menstrual dates and clinical landmarks. Caution must be exercised before any "changing" of dates with ultrasound findings. What clinical data are available should confirm the ultrasonic findings, and even then the most conservative dates should be selected. Third-trimester scans should not be used to change dates, as one risks occurrence of respiratory distress syndrome in a less mature infant with a higher biparietal diameter percentile. Such a deviation from protocol was noted by Frigoletto et al.[14] In contrast, the use of clinical parameters alone by Harris and Meade[27] resulted in a 3% incidence of respiratory distress syndrome.

A glucose screen was performed because infants of diabetic mothers may be large for estimated dates, giving a false clinical and ultrasound impression of further gestational age than dates indicate. At the same time lung maturity may be delayed in a diabetic pregnancy.[28] One Class B diabetic patient in this series with good estimated dates required three amniocenteses before a mature value was obtained at 41 weeks. It is therefore still recommended that an amniocentesis for indices of pulmonary maturity still be performed before elective scheduling in the diabetic patient.

The use of the terminal ultrasound examination and the biparietal diameter value of ≥9.2 cm exists in the management scheme for several reasons. First, unpublished experience over several years at this institution suggested that if gestational age had been confirmed with ultrasound scans during the second trimester, then a biparietal diameter of ≥9.2 cm after 38 weeks was always associated with pulmonary maturity. Published data[19] showed retrospectively that in the absence of diabetes no cases of respiratory distress syndrome occurred if the biparietal diameter was ≥9.2 cm, although lung profiles were transitional in 4 of 57 cases. Second, management involving only clinical landmarks and midtrimester ultrasound studies[14] had still resulted in a small incidence of iatrogenic prematurity with respiratory distress syndrome. Third, the terminal examination provided a late assessment of fetal growth and an additional buffer against error in assessment of gestational age. It pointed out suspected intrauterine growth retardation or possible minor dating error and suggested antepartum testing, amniocentesis, indicated delivery, or simple delay in scheduling in several cases.

On the other hand, it is felt that the terminal examination cannot be used in a vacuum for all the reasons already discussed, including diabetes, wide dating error of third-trimester scans, and the inability of the biparietal diameter alone to predict the lethicin/sphingomyelin ratio.[18] In the two cases with 9.6 cm biparietal diameters and lethicin/sphingomyelin ratios <2.0 : 1 (1.8 and 1.9), there were no dates supporting clinical landmarks because of late registration. (In both cases, values of optical density at 650 nm[29] were mature.) The biparietal diameter value of ≥9.5 cm was chosen as a more conservative ultrasonic indicator of term in the absence of known last menstrual period or last menstrual period–supporting data. However, an ultrasound estimated date of confinement with supporting landmarks is still required (Fig. 1, *B*).

Thirty-nine weeks is used as the time for scheduling because it potentially leaves the majority of patients available for elective delivery prior to labor (here it was 76.3%), yet at the same time provides a fair margin of safety in terms of gestational age, considering most clinical and ultrasound parameters have an accuracy of ± 10 days to 2 weeks.

The high incidence of "unknown" (usually suspected by patient history and prior cesarean section indication, but undocumented for the most part) scars again relates to the high degree of mobility in the study population. Prior cesarean sections had been performed in multiple civilian and military hospitals around the world. Both late registrations and the relative isolation of our institution in Hawaii additionally made the obtaining of scar documentation most difficult.

Finally, in terms of performance the management protocol did succeed in eliminating iatrogenic prematurity in electively scheduled repeat cesarean sections. At the same time it resulted in the performance of amniocentesis in only 13.2% of the cases. This contrasts with the 32.1% incidence in Toffle et al.'s series[15] or the 28.5% incidence in Hayashi et al.'s series[17] and compares with the 12.7% in Frigoletto et al.'s series[14]. Furthermore, it did this without decreasing the numbers of patients scheduled electively on protocol criteria alone (54.2%) or delivered as planned (46%) versus the results of Toffle et al. of 59.5% and 50.8%, respectively. With better patient compliance and logistic support an even greater number could have been safely delivered on a scheduled elective basis with the use of this combined systems approach to scheduling.

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Previous cesarean section: The risks and benefits of oxytocin usage in a trial of labor

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Permitting a trial of labor in patients with a previous cesarean birth is rapidly becoming an accepted alternative to routine elective repeat cesarean section. As interest in this approach has grown and the risks associated with a trial of labor have been better defined, the use of oxytocin in these patients emerges as a pertinent issue. Our retrospective experience of oxytocin use in patients undergoing a trial of labor suggested no increased maternal or fetal risk compared to patients who did not receive oxytocin. On the basis of our prior experience, we set out to investigate prospectively the role of oxytocin in 732 patients with prior cesarean section who underwent a trial of labor. During the study period, 289 (40%) patients received oxytocin for either induction (32, 11%) or augmentation (257, 89%) of labor and 443 patients did not receive oxytocin. Successful vaginal delivery was achieved in 200 patients (69%) as opposed to 395 (89%) of the patients who did not receive oxytocin. The incidences of dehiscence in the oxytocin and no oxytocin groups were 3% and 2%, respectively. Further analysis of vaginal and cesarean delivery complications for the two groups were contrasted and no significant differences were found with respect to the incidence of hemorrhage, uterine atony, hysterectomy, or the requirement of transfusions. Neonatal outcome was also comparable for both groups. On the basis of our prospective experience, it appears that the use of oxytocin, when carefully monitored, is a safe and reasonable consideration in the patient undergoing a trial of labor. (AM J OBSTET GYNECOL 1985;151:564-9.)

Key words: Cesarean section, trial of labor, oxytocin

Recent clinical attention has focused upon whether patients with a prior cesarean delivery should be permitted a trial of labor. Several investigators have reported that vaginal delivery after cesarean section is safe when done in properly selected patients and in the appropriate clinical setting.[1-11] These investigators concluded, therefore, that a trial of labor does appear to be a reasonable alternative to routine repeat cesarean delivery.

Although the current literature attests to the merits of a trial of labor in the patient with prior cesarean section, several major clinical questions have not been adequately addressed in the past. For example, can patients undergoing a trial of labor receive oxytocin? The major argument against oxytocin use has been that the intrapartum administration of oxytocin would be associated with an increasd risk of uterine rupture and its attendant maternal and fetal sequelae.[12-14] However, prior reports[1-6] and our own retrospective experience[15] on the use of oxytocin in patients undergoing a trial of labor have failed to demonstrate any increased maternal or fetal risk. Nonetheless, and because the total population studied to date is relatively small, a prospective investigation was undertaken at our institution to clarify further the risks and benefits of oxytocin administration in patients with a prior cesarean birth who undergo a trial of labor.

Material and methods

Between July 1, 1982, and June 30, 1983, patients with prior cesarean births were prospectively studied at the Los Angeles County/University of Southern California Medical Center. During this time period, patients with a known vertical incision or two or more prior cesarean sections were not permitted a trial of labor. In addition, and for purposes of this report, patients with multiple gestations, unknown intent as to a trial of labor, and a previous hysterotomy or myomectomy were excluded. Prior to an attempted trial of labor and the administration of oxytocin, informed consent was obtained.

In those patients undergoing a trial of labor, oxytocin was administered when indicated for obstetric reasons. Oxytocin was administered intravenously in an incremental fashion to a maximum dose of 22 mU/min via a Harvard pump. Patients undergoing a trial of labor

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Table I. Indication for the use of oxytocin in patients with previous cesarean section

Indication	*Total (N)*	*Vaginal delivery*	
		N	*%*
Induction of labor	32	23	72
Augmentation of labor	257	177	69
Prolonged latent phase	17	14	82
Prolonged active phase	6	6	100
Arrest of dilatation	107	49	46
Failure to descend	5	4	80
Protracted descent	11	11	100
Arrest of descent	3	0	0
Desultory labor	50	43	86
Reason for use unclear	58	50	86

Table II. Delivery outcome for 732 patients with prior cesarean section with regard to use of oxytocin and indications for repeat cesarean section

Indication for cesarean delivery	*Oxytocin, cesarean delivery (N = 89, 31%) (n)*	*No oxytocin, cesarean delivery (N = 48, 11%) (n)*
CPD/FTP	55	28
Fetal distress	8*	11*
Breech	3	4
Maternal	11	0
Other	12	5

CPD/FTP = Cephalopelvic disproportion. Vaginal delivery, oxytocin, 200 (69%) and vaginal delivery, no oxytocin, 395 (89%): $p < 0.05$.

*$p < 0.05$.

were continuously monitored with direct fetal monitoring techniques. Clinical findings consistent with uterine rupture were considered indications for immediate abdominal delivery. Otherwise, cesarean section was reserved for obstetric indications.

Maternal outcome for the oxytocin and nonoxytocin groups were assessed as to successful trial of labor, previous indication for cesarean delivery, uterine dehiscence or rupture, febrile morbidity, blood loss at delivery, transfusion requirements for each group, and the duration of hospital stay. Fetal and neonatal outcomes for the two groups were also contrasted as to the incidence of Apgar scores <7 at 1 and 5 minutes, birth weight, and perinatal mortality.

Statistical analysis was done with the use of χ^2 analysis and Student's *t* test. Significance was considered with a p value <0.05.

Results

During the study period, 732 patients underwent a trial of labor. Of these, 289 (40%) received oxytocin for either the induction (32) or the augmentation (257) of labor. Indications for oxytocin are illustrated in Table I. In those patients who received oxytocin, for either induction or augmentation of labor, a similar incidence of vaginal deliveries was observed. In patients receiving oxytocin for induction of labor (n = 32, 11%), 23 (72%) were deliverd vaginally and nine (28%) were delivered by cesarean section. In patients receiving oxytocin for augmentation of labor (n = 257, 89%), 80 (31%) were delivered by cesarean section and 177 (69%) were delivered vaginally. The mean duration of oxytocin was 6.9 ± 5.7 hours.

Table III. Vaginal delivery complications associated with trial of labor and separated as to oxytocin usage

Complication	*Oxytocin (N = 200)*		*No oxytocin (N = 395)*	
	n	*%*	*n*	*%*
Perineal lacerations (third/fourth degree)	30	15	62	16
Hemorrhage (>500 ml)	37	19	61	15
Uterine atony	9	5	8	2
Transfusion	9	5	12	3

Table IV. Intraoperative complications associated with cesarean delivery and separated as to oxytocin usage

Complication	*Oxytocin (N = 89)*		*No oxytocin (N = 48)*	
	n	*%*	*n*	*%*
Hemorrhage (>1000 ml)	10	11	9	19
Uterine atony	3	3	1	2
Lacerations/incision extension	0		2	4
Bladder injury	2	2	1	2
Hysterectomy	1	1	1	2
Transfusion	8	9	8	17

In Table II, outcome of the trial of labor for the two study groups is depicted. Successful vaginal delivery was significantly more frequent in the nonoxytocin group (89%) than in the oxytocin group (69%) ($p < 0.05$). With the exception of fetal distress, indications for repeat cesarean birth were similar for both groups. In the nonoxytocin group, operative intervention for fetal distress occurred significantly more often than in those patients who received oxytocin ($p < 0.05$).

The vaginal delivery complications associated with a trial of labor, separated as to oxytocin usage, are illustrated in Table III. The incidences of perineal lacer-

Table V. Maternal outcome and hospital stay for those patients undergoing a trial of labor with or without oxytocin

	Oxytocin (N = 289)				*No oxytocin (N = 443)*			
	Success (N = 200)		*Failure, cesarean section (N = 89)*		*Success (N = 395)*		*Failure, cesarean section (N = 48)*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Febrile morbidity	4	2	24	27	9	2.3	12	25
Dehiscence	6	3.0	3	3.4	5	1.3	1	2.1
Hospital stay (days)	2.3		4.1		2.2		4.6	

Table VI. Prior cesarean section neonatal outcome

	No oxytocin (N = 433)				*Oxytocin (N = 289)*			
	Vaginal delivery (N = 395)		*Cesarean delivery (N = 48)*		*Vaginal delivery (N = 200)*		*Cesarean delivery (N = 89)*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
1 min Apgar score <7	43	11	19	40	32	16	31	34
5 min Apgar score <7	3	0.8	5	10	4	.2	5	6
Antepartum death	1		0		1		1	
Neonatal death	1		0		0		1	
Mean birth weight (gm)	3429		3695		3420		3639	

ations, hemorrhage >500 ml, uterine atony, and the need for blood transfusions were comparable for both groups.

Table IV illustrates the intraoperative complications associated with cesarean delivery in those patients undergoing a trial of labor separated as to oxytocin usage. Both groups were comparable with respect to the incidence of uterine atony, bladder injuries, and need for hysterectomy; however, a marked, but insignificant increase in hemorrhage >1000 ml, the need for blood transfusion, and lacerations or extensions of the incision was found in the group that did not receive oxytocin. In the two patients who underwent hysterectomy, the indication was atony in both instances. Neither patient had a uterine dehiscence.

For those patients undergoing a trial of labor, the outcome as it relates to febrile morbidity, dehiscence, and hospital stay is illustrated in Table V. When the oxytocin and no oxytocin groups are contrasted, the incidences of febrile morbidity and hospital stay were found to be similar. While the dehiscence rate in the oxytocin group was higher than that in those who did not receive oxytocin, these results were not significantly different. In those patients with a dehiscence at delivery, two patients had complications that warrant comment. The first case occurred in the oxytocin group and has been described previously.[16] In that case, the patient with a prior cesarean section for cephalopelvic disproportion presented in labor at 42 weeks' gestation. Subsequently, she required oxytocin augmentation and later experienced arrest of descent at 8 cm. Prior to the cesarean section, the infant's head was manually disengaged from the pelvis via the vagina. At cesarean delivery, the infant was found partially extruded, to the level of the shoulders, through a dehiscent low-transverse uterine incision. The Apgar scores were 2 and 9 at 1 and 5 minutes, respectively. The dehiscence was repaired. Maternal and neonatal courses were uneventful. The second patient, a 28-year-old woman, gravida 4, para 2-0-1-2, with a prior cesarean delivery for hemorrhage, was in the nonoxytocin group. She presented in labor with an unknown type of uterine scar at term and was allowed a trial of labor. Subsequently, she was delivered vaginally of a 3700 gm infant with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. However, because of a retained placenta and persistent postpartum bleeding, the diagnosis of uterine rupture or placenta accreta was considered. Exploratory laparotomy was done with the finding of a uterine rupture of the prior transverse fundal incision. In addition, there was clinical evidence to suggest placenta accreta at the rupture site. The edges of the rupture were debrided and then reapproximated. The maternal postoperative course was uneventful.

Neonatal outcome for the study population is displayed in Table VI. The incidence of Apgar scores <7 at 1 and 5 minutes, respectively, and the mean birth weights were comparable in both study groups. Furthermore, there were three antepartum and two neonatal deaths. The fetal deaths were all unrelated to a trial of labor as all three patients either presented in active labor or underwent induction of labor because of the finding of an antepartum stillbirth. Similarly, the neonatal deaths were also unrelated to the trial of labor.

Table VII. Previous indication for cesarean birth and subsequent trial of labor separated as to oxytocin usage

	Oxytocin			*No oxytocin*		
		Vaginal delivery			*Vaginal delivery*	
Indication	*N*	*n*	*%*	*N*	*n*	*%*
CPD/FTP	150	98	65*	177	149	81
Breech	49	41	84	86	81	94
Fetal distress	29	16	55*	42	41	98
Other	61	45	74	138	124	90

CPD/FTP = Cephalopelvic disproportion/failure to progress.
*$p < 0.05$.

Table VIII. Effect of vaginal birth either prior to or after cesarean delivery on subsequent use of oxytocin during trial of labor

	Oxytocin			*No oxytocin*		
		Vaginal delivery			*Vaginal delivery*	
Vaginal birth	*N*	*n*	*%*	*N*	*n*	*%*
Prior to cesarean delivery						
None	216	141	65*	336	302	90
≥1	68	56	82	101	88	87
After cesarean delivery						
None	254	170	67*	346	303	88
≥1	29	26	90	90	86	96

*$p < 0.05$.

The neonatal death in the no oxytocin group occurred in a patient who was delivered of a 3140 gm anencephalic infant that subsequently died on the fourth day of life. The other patient in the oxytocin group was delivered by cesarean section for a persistent fetal heart rate bradycardia. The infant weighed 2700 gm and had multiple congenital anomalies, which included transposition of the great vessels. Apgar scores were 2 and 5 at 1 and 5 minutes and the infant subsequently died.

Additional factors, namely, the previous indication for cesarean delivery and the effect of a prior vaginal birth, were evaluated to determine their effect on a subsequent trial of labor. As shown in Table VII, patients with a previous indication for cesarean section of fetal distress or cephalopelvic disproportion were significantly less likely to be able to achieve a vaginal delivery if they received oxytocin in the subsequent pregnancy. Further evaluation of those 13 patients with a prior cesarean section for fetal distress and who received oxytocin demonstrated that the reasons for the repeat cesarean section were cephalopelvic disproportion in eight, fetal distress in two, and other in three.

The effect of the number of vaginal births on the subsequent use of oxytocin during a trial of labor was analyzed and is demonstrated in Table VIII. Those patients who had had a vaginal birth had the greatest likelihood of a successful trial of labor whether or not oxytocin was administered. Of importance, those patients who had not experienced a vaginal birth had a greater likelihood of undergoing a repeat cesarean delivery ($p < 0.05$). However, despite this difference, two thirds of those patients without a prior vaginal birth and who did require oxytocin still achieved a vaginal delivery.

Comment

Contrary to previous reports,[12-14] the use of oxytocin in patients undergoing a trial of labor with a prior cesarean delivery is not associated with an increasd incidence of uterine rupture. In those series detailing oxytocin usage, the incidence of uterine dehiscence or rupture has been reported to range from 1% to 8% (Table IX).[1-6, 15, 19] The dehiscence rates in our two studies are consistent with those of these reports. When the results were contrasted with those in a group of patients who did not receive oxytocin, no significant difference was found. Furthermore, none of the dehiscences in either group led to any major maternal or fetal sequelae.

More important, the judicious use of oxytocin was not associated with an increase in maternal morbidity. As demonstrated by the current report, the use of oxytocin was not associated with an increased incidence of blood loss at delivery, transfusions, or hysterectomy. This is in contrast with our previous report, which showed a greater incidence of blood loss and transfusions in the oxytocin group.[15] This difference can be

Table IX. Reports of uterine dehiscence in patients with previous cesarean section receiving oxytocin

Series	*No. of patients undergoing trial of labor*	*Oxytocin usage*		*Uterine dehiscence*	
		n	*%*	*n*	*%*
Riva and Teich,[1] 1961	214	7	3.3	0	0
McGarry,[2] 1969	274	83	30	1	—
Meehan et al.,[3] 1972	75	25	33	2	8
Saldana et al.,[4] 1979	145	3	2	0	0
Demianczuk et al.,[5] 1982	42	23	25	2	8
Martin et al.,[6] 1983	162	25	17	0	0
Horenstein et al.,[15] 1984	292	58	20	3	5
Flamm et al.,[19] 1984	230	94	41	1	1
Current study	732	289	40	9	3
Total	2166	607	28	28	4.6

Table X. Reports of successful vaginal delivery in patients with previous cesarean section receiving oxytocin

	Oxytocin			*No oxytocin*		
	No. of patients	*Vaginal delivery*		*No. of patients*	*Vaginal delivery*	
		n	*%*		*n*	*%*
McGarry,[2] 1969	83	51	62	191	145	76
Saldana et al.,[4] 1979	3	3	100	142	51	38
Demianczuk et al.,[5] 1982	23	10	43	69	40	58
Meier and Porreco,[7] 1982	42	34	81	164	141	86
Horenstein et al.,[15] 1984	58	31	53	234	186	84
Flamm et al.,[19] 1984	94	61	65	136	120	88
Current study	289	200	69	443	395	89
Total	592	390	66	1279	1078	84

explained by the larger study population in the current series. Like the maternal outcome, neonatal outcome was similar for both groups.

In the current report, vaginal delivery occurred significantly less often in those patients who received oxytocin (Table II). While these results were lower for the oxytocin group, 69% still achieved a vaginal delivery. Of note, a 30% increase in successful vaginal deliveries was observed in our second study period (Table X). This difference is in all likelihood related to feeling more comfortable with the use of oxytocin in the patient with prior cesarean section undergoing a trial of labor and is reflected by the 94% increase in its use in the 1982 and 1983 study period.

As previously noted by Clark et al.,[17] patients with a prior cesarean birth for cephalopelvic disproportion were less likely to achieve a vaginal delivery, whereas those patients with a prior cesarean section for breech presentation were more likely to be delivered vaginally. A similar relationship was observed in the current report (Table VII). Furthermore, if oxytocin was used during the subsequent pregnancy, the likelihood of achieving a vaginal delivery was less for all patients, irrespective of the previous indication for cesarean section. Why patients with a previous indication of fetal distress for cesarean section were more likely to undergo a repeat cesarean section when oxytocin was administered is unclear. At the same time, it is important to stress that the indication for repeat cesarean section in this group was for reasons other than fetal distress.

Last, patients with a prior vaginal birth are more likely to achieve a vaginal delivery.[18] The current report further corroborates that finding with the greatest degree of success in those patients with a vaginal birth after a previous cesarean section who have not received oxytocin.

On the basis of our experience, we believe that oxytocin may be safely used in patients with a previous cesarean delivery who undergo a trial of labor. Oxytocin may be used for the usual obstetric indications in the patient with a prior cesarean birth provided that the labor and the fetus are closely monitored.

We wish to thank Fidelia Diaz, M.A., for gathering the information, and Sze-Ya Yeh, M.D., for his computer assistance.

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Labor risk assessment in a rural community hospital

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Unanticipated perinatal catastrophe in a rural community hospital is responsible for the deaths of many potentially salvageable infants. An intrapartum risk scoring system was designed to help anticipate infants needing skilled resuscitation at the rural community hospital. Thirty-three percent of infants whose mothers scored as high risk (≥10) required resuscitation while only 6% of infants whose mothers scored <10 required resuscitation. Sequential Apgar scores of infants in need of resuscitation attended by a skilled resuscitator showed significant improvement compared with Apgar scores of infants not attended by a skilled resuscitator, suggesting at least a short-term benefit for early identification of the infant at risk. (AM J OBSTET GYNECOL 1985;151:569-74.)

Key words: Labor, resuscitation, Apgar scores

Regionalization of perinatal care and improved obstetric management has lowered neonatal mortality in the United States from 15.1 neonatal deaths per 1000 live births in 1970 to 7.6 neonatal deaths per 1000 live births in 1982.[1] In a 1981 summary of neonatal mortality statistics, 90% of this mortality was attributed to asphyxia, congenital anomalies, respiratory distress syndrome, bacterial sepsis, and birth weight ≤750 gm, all of which may lead to the unexpected low–Apgar score infant.[2] Asphyxia alone is responsible for 23.5% of neonatal deaths in Level I hospitals compared to 3% of neonatal deaths occurring in the Level III hospital. Since much of the preventable perinatal mortality and morbidity occurs in the community hospital, an easily applied, reliable method that predicts the low–Apgar score infant in that setting is required. Once risk is identified, resources can then be mobilized for appropriate intervention.

A variety of antepartum scoring systems that focus on prenatal risk have been developed at tertiary centers.[2-7] However, we also need to identify and plan for that group of patients that are at low risk prior to delivery and subsequently become high risk in the intrapartum period. This group of patients may contribute the most to otherwise preventable perinatal mortality and morbidity in the Level I hospital.

Little information directly applicable to intrapartum risk assessment in a rural perinatal center was found;

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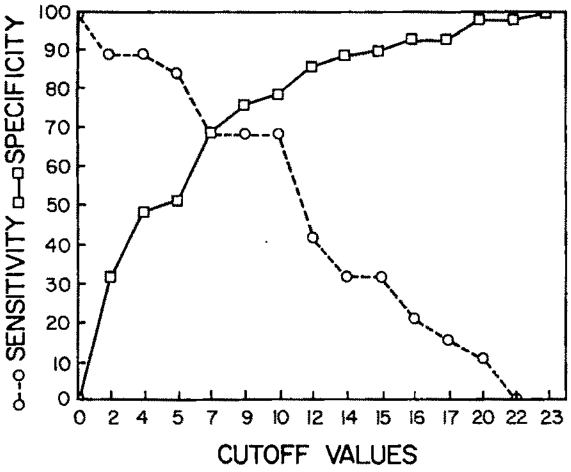

Fig. 1. Effect of varying cutoff value on sensitivity and specificity of risk score.

therefore, this study was undertaken to develop, on the basis of available literature and our own local rural perinatal experiences, a risk scoring system that would reliably predict the low–Apgar score infant. A retrospective chart review was done to assess the reliability and predictability of the labor risk scoring system.

Material and methods

Sample. Memorial Hospital of Carbondale is a 151-bed community hospital in rural southern Illinois. There are approximately 1200 deliveries per year. The population is 89% Caucasian and the majority of patients are socioeconomically lower to middle class with 33% on public aid. Forty-three percent of the patients are primiparous.

Located at Memorial Hospital is a Level II Special Care Nursery serving all of southern Illinois. Ten percent of the inborn infants require transfer for special care. At the time of this study, attendance at delivery by the neonatologist was at the request of the attending obstetrician, pediatrician, or family physician. All cesarean sections and deliveries complicated by meconium-stained amniotic fluid were attended by a physician. Infants were otherwise routinely attended by an obstetric nurse.

Procedure. One hundred forty obstetric and newborn charts from October, 1981, through February, 1982, were randomly selected from 451 deliveries to evaluate an ad hoc additive system of intrapartum risk assessment for the low–Apgar score infant. Factors were included and weighted values were assigned on the basis of previous studies and local clinical experience (Table I). A high-risk score was defined as a sum score ≥10. Risk was assumed to be additive. One hundred charts were reviewed for presence of risk factors. A labor risk score was then assigned. Delivery information included type and time of delivery, 1- and 5-minute Apgar scores, presence of a skilled resuscitator (one capable of intubation and biochemical resuscitation), and neonatal outcome. Forty deliveries were scored prospectively at the bedside by labor and delivery nurses to test the practical design of the scoring sheet.

Table I. Factors used for determination of risk score with assigned weights.

Risk factor	*Weighted value*
Baseline data	
Age <17 yr or >34 yr	2
Parity ≥4	2
Premature labor	
36-38 wk	5
≤35 wk	10
Postmature labor (>42 wk)	5
Medical history	
Diabetes	2
Requiring insulin	5
Hypertension (chronic)	2
Gestational (>20 mm Hg) or preeclampsia	5
Eclampsia	10
Renal	2
Endocrine	2
Cardiovascular	2
Infectious disease (herpes virus, venereal disease, hepatitis)	2
Outbreak within last 3 wk	5
Epilepsy	2
Smoker	2
Excessive alcohol intake	5
Drug abuse	5
Medications during labor	
Within 2 hr of delivery	5
Past obstetric history	
Previous perinatal death	5
Previous premature or low–birth weight infant	5
Blood incompatibility (or previous jaundiced infant)	5
≥2 Spontaneous abortions	5
Current pregnancy	
Antepartum hemorrhage	5
Prolapsed cord	10
Meconium (nonbreech)	10
Fetal distress	10
Eclampsia or severe preeclampsia	10
Abnormal presentation	2
Prolonged rupture of membranes (>24 hr)	5
Multiple gestation	5
Prolonged second stage (≥2.5 hr)	5

Fetal distress was defined as either (1) persistent fetal bradycardia (<120 bpm), (2) persistent fetal tachycardia (>180 bpm), (3) severe variable or late decelerations, or (4) loss of beat-to-beat variability. All monitor strips were later reviewed to confirm the clinical diagnosis of fetal distress but the clinical assessment was the one used in assigning risk scores.

Table II. Performance of labor risk score in predicting need for resuscitation

Predicted risk	*1 min Apgar score ≤5* %	*1 min Apgar score ≤5* n	*p Value*	*Sensitivity (%)*	*Specificity (%)*	*False positive (%)*	*False negative (%)*
Low (score <10)	6	6/101	<0.0001	68	79	67	6
High (score ≥10)	33	13/39					

N = 140.

Table III. Details on the 19 infants requiring resuscitation

Risk factor	*Risk score*	*Apgar score* 1 min	*Apgar score* 5 min	*Attended*
Postmature, fetal distress, prolonged second stage	20	2	3	No
Fetal distress, meconium	20	3	8	Yes
Gravida 4, prematurity, PROM	17	5	7	No
Prematurity, breech, smoker, h/o VD	16	3	8	Yes
Prematurity, antepartum hemorrhage	15	1	6	Yes
Hypertension, meconium	15	2	7	Yes
Prematurity, breech	12	3	5	No
Postmaturity, drug use, smoker	12	1	6	No
Prematurity	10	4	7	Yes
Meconium	10	5	9	Yes
Prematurity, prolonged second stage	10	4	7	No
Fetal distress	10	5	7	No
Fetal distress	10	3	6	No
Postmaturity	5	5	6	No
Hypertension	5	5	9	No
Hypertension	5	5	8	No
Smoker, breech	4	3	6	No
None	0	5	8	No
None	0	5	9	No

PROM = Premature rupture of membranes; h/o VD = history of venereal disease.

Need for skilled resuscitation was defined by a 1-minute Apgar score ≤5.

Statistical analysis. Contingency table analysis and *t* tests were used to assess the association between Apgar scores and individual risk factors. The standard χ^2 test was used to determine statistical significance of the tables meeting the cell size assumptions of this test. For tables with small cell counts (one or more cells with expected cell count <3) the maximum likelihood ratio χ^2 was used. Multiple least-squares regression and logistic regression analyses were used to investigate the joint association of multiple risk factors and Apgar scores.[8]

Sensitivity was defined as the percent of infants in need of resuscitation (Apgar score ≤5) who were correctly scored as needing resuscitation (risk score ≥10).

Specificity was defined as the percent of infants not needing resuscitation who were correctly scored as not needing resuscitation.

False positive results included those infants classified as high risk but not requiring resuscitation. False negative results included those infants classified as low risk but requiring resuscitation.

Results

A total of 140 patients were assigned scores, and 39 (28%) scored as high risk. Thirteen of the 39 infants in the high-risk group required resuscitation (33%). Six of the 101 infants in the low-risk group required resuscitation (6%). This was statistically significant by the χ^2 test ($p < 0.0001$). Sensitivity was 68% and specificity was 79%. False positive results occurred in 26 of 39 (67%). False negative results occurred in six of 101 (6%) (Table II).

When the definition of the cutoff value for the high-risk score was varied from 5 to 20, the optimal balance of high sensitivity and low false negative rate was at the initial empirically assigned value of 10 (Fig. 1).

Details of the 19 infants with 1-minute Apgar scores ≤5 are shown in Table III. Risk factors occurring singly and in combination included premature labor (six), fe-

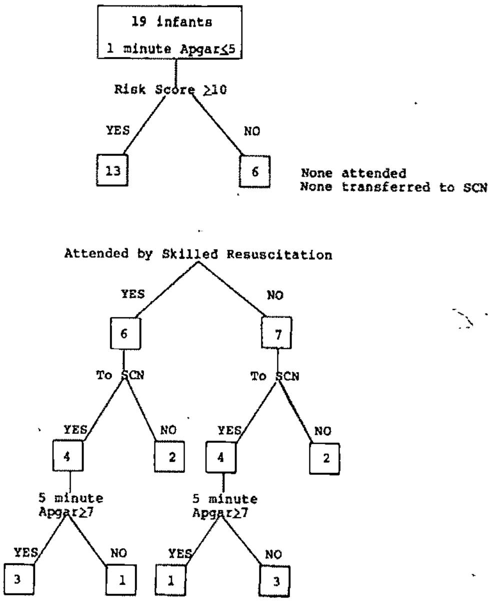

Fig. 2. Immediate postnatal care of the 19 infants requiring resuscitation.

Table IV. Mean change in Apgar score between 1 and 5 minutes when delivery attended by skilled resuscitator for 19 infants requiring resuscitation

	Attended		*Not attended*	
Predicted	*Mean change*	*n*	*Mean change*	*n*
High risk (N = 13)	4.5	6	2.6*	7
Low risk (N = 6)	–	0	3.0	6

*Significantly lower than mean for attended infants (*t* test, $p < 0.01$).

tal distress (four), postmaturity (three), gestational hypertension (three), meconium-stained amniotic fluid (three), abnormal presentation (three), and prolonged second stage of labor (two). All cases were considered low risk until the intrapartum period. There were no deaths.

Thirteen low–Apgar score infants were correctly predicted. Four out of six attended high-risk infants required admission to the Special Care Nursery (67%). Four of the seven high-risk infants with unattended deliveries required admission to the Special Care Nursery (57%). None of the six false negative infants required admission to the Special Care Nursery (Fig. 2).

Average improvement in Apgar scores of the 19 high-risk infants is shown in Table IV. Apgar scores improved 4.5 points between 1 and 5 minutes in high-risk infants with attended deliveries as compared to a 2.6 point increase for those high-risk infants with deliveries not attended by a skilled resuscitator. Mean 1-minute Apgar scores were slightly lower in the attended group (3.0 versus 3.3), although not statistically different (*t* test, $p > 0.7$).

Table V. Risk factors versus Apgar score

Factor	*No.*	*1 min Apgar score ≤5 (%)*	*p Value*
Abnormal presentation	4	75	$p < 0.005$
Premature labor, ≤35 wk	7	71	$p < 0.001$
Hemorrhage	2	50	NS
Prolapsed cord	2	50	NS
Premature labor, <38 wk	14	43	$p < 0.001$
Prolonged second stage	5	40	NS
Fetal distress	11	36	$p < 0.05$
Risk score ≥10	39	33	$p < 0.0001$
Meconium staining	10	30	NS
Poor obstetric history	19	0	$p < 0.07$
Total, all patients	140	14	–

Table V displays the results of the contingency table analysis relating individual antenatal and intrapartum factors to the 1-minute Apgar score. Although numbers are small, low 1-minute Apgar scores were more frequently associated with abnormal presentation, prematurity, fetal distress, and a risk score ≥10. Past obstetric history was negatively correlated with low 1-minute Apgar score and falsely elevated the risk score. Removing this factor from the analysis improved specificity from 79% to 85% without altering sensitivity.

On the basis of these results and multiple logistic regression analysis relating the joint effect of risk factors to Apgar scores, a shortened index of five factors was tested for predictive value against 1-minute Apgar score. Results were compared with those from analysis of the scoring system as originally designed. The factors included were premature labor ≤35 weeks, abnormal presentation, fetal distress, prolonged second stage of labor, and meconium staining. Six infants of 109 patients with none of these characteristics had a 1-minute Apgar score ≤5 (5.5%). Thirteen infants of 31 patients with at least one of these factors had a low 1-minute Apgar score (42%) (Table VI).

Comment

A major goal of perinatal medicine is prevention rather than treatment of disease. This is the most effective way to reduce mortality and most likely morbidity. This goal is attained by risk assessment and subsequent management that either eliminates or reduces risk to both mother and infant. It is essential to have numerical indices of risk that are accurate and repeatable and, when significant intervention is projected, carry the absolute minimum of false negative results.

This study assessed the usefulness of an empirical intrapartum risk scoring system developed to help pre-

Table VI. Performance of shortened index in predicting need for resuscitation

Index	*1 min Apgar score ≤5*		*p Value*	*Sensitivity (%)*	*Specificity (%)*	*False positive (%)*	*False negative (%)*
	%	*n*					
Negative	5.5	6/109	<.0001	68	85	58	6
Positive	42	13/31					

N = 140.

dict infants who may need skilled resuscitation. Sensitivity and specificity of this scoring system compare favorably with those of other risk assessment scores used in tertiary care centers.[9-13] A higher than expected number of patients were classified as high risk causing a false positive rate of 67%.

The initial scoring system included all risk factors previously shown in multiple large series to correlate with increased perinatal mortality. Several factors were not significantly related to Apgar score and therefore could be either excluded or weighted less. These included previous poor obstetric history, previous perinatal death, previous premature or low–birth weight infant, previous jaundiced infant, smoking, gestational hypertension, or two or more spontaneous abortions. These nonsignificant factors falsely elevated scores, thereby placing some mothers who had normal outcomes into the high-risk category.

Appropriate prepartum management of the above high-risk mothers may avoid unsatisfactory intrapartum outcomes and thus decrease the power of these variables to predict risk. As unsatisfactory outcomes are avoided by appropriate intrapartum management, for instance, routinely attending deliveries associated with meconium staining of amniotic fluid, cases will be recorded as false positive, further sacrificing index effectiveness. The low false negative rate of 6% reflects the conservative clinical approach necessary to maximize positive infant outcome.

Fetal distress was diagnosed in 11 cases. Retrospective review of these tracings confirmed the clinical impression in six cases. None of the five unconfirmed cases required resuscitation. Use of fetal scalp pH determinations to evaluate worrisome or questionable fetal heart rate patterns may further improve the predictive power of this variable. These data were not available for the 11 cases reviewed in this study.

Review of the Apgar score data demonstrates the importance of a skilled resuscitator at a projected high-risk delivery. Mean 5-minute scores were significantly greater in the attended high-risk group. Although this analysis of Apgar scores is presented as if an interval scale applied, in clinical practice the interpretation of the Apgar score is not linear. The increase from 3.0 to 7.5 in the attended group may therefore be even more important to the infant than the numbers reflect.

The miniindex of five factors proved superior in predictive value when compared to the complete labor risk score. However, with the small number of patients reviewed and the low incidence of intrapartum catastrophic events in otherwise low-risk pregnancies, further application on a larger population is needed before either the miniindex or complete labor risk score can be recommended for all rural Level I hospitals in our regional perinatal network. A hospital or region interested in developing a similar scoring system would need to include or exclude variables on the basis of their own clinical experience and expertise and validate their risk assessment scale in a similar matter.

Unlike previous published scoring systems, our system was designed to help the obstetric staff of a rural community hospital assess any given pregnancy for the presence of increased intrapartum risk for the fetus. In its present form the labor risk score is a reasonably reliable, repeatable, and clinically useful predictor of the infant in need of resuscitation. The miniindex seems an even more powerful predictor of the low–Apgar score infant. With this early information, there should be sufficient time to consult, transfer, or assemble a skilled resuscitation team for the anticipated high-risk delivery and thereby minimize the potentially disastrous consequences of the unanticipated delivery of a distressed newborn infant.

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Prevention of premature labor by 17α-hydroxyprogesterone caproate

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Eighty pregnant women at high risk of giving birth prematurely were divided randomly into two groups. Treatment with either 17α-hydroxyprogesterone caproate, 250 mg by intramuscular injection once a week, or a placebo was given in a double-blind fashion. Imminent premature labor occurred in 29.0% of the treated group and in 59.4% of the control group ($p < 0.025$). The rate of premature deliveries was also significantly lower in the treated group (16.1%) than in the control group (37.32%) ($p < 0.05$). There were no cases of perinatal death or fetal malformations in either group. The mean birth weight of all infants of the treated group was significantly higher than in those of the control group (3111.9 ± 905 gm versus 2680 ± 813.4 gm, $p < 0.05$). The results support treatment with progesterone caproate for the prevention of premature labor. (AM J OBSTET GYNECOL 1985;151:574-7.)

Key words: Prevention of premature labor, 17α-hydroxyprogesterone caproate

The most common cause of neonatal death is low birth weight, usually as a consequence of prematurity.[1] Patients at high risk of giving birth prematurely can be identified at an early stage.[2,3] The challenge facing the physician then is to prevent premature labor by means of long-term treatment starting early in pregnancy.

Johnson et al.[4] in their first double-blind study and in their second study,[5] which was not double-blind, found that the number of premature deliveries in a high-risk population could be reduced by means of weekly injections of 17α-hydroxyprogesterone caproate.[4,5] Not much additional evidence for the clinical effectiveness of this agent has been accumulated to date, thus "confirmation of their observation is awaited."[1] Johnson et al.[5] concluded that "much larger studies will be required to delineate the true risk/benefit ratio of such a treatment."

The present study provides further evidence of the effectiveness of 17α-hydroxyprogesterone caproate in preventing premature labor in high-risk patients.

Material and methods

Eighty pregnant women in whom the current pregnancy had been immediately preceded by at least two preterm deliveries or two spontaneous miscarriages or a combination of both were included in the study. Patients with multiple pregnancies, diabetes, chronic renal disease, and chronic hypertension were excluded. An ultrasound examination was performed upon referral in each case in order to confirm embryo viability and to determine gestational age. After giving informed consent, patients were randomly divided into two groups according to the last digit of their clinical registration number. Until gestation week 37, half of the patients were given weekly intramuscular injections of 250 mg of 17α-hydroxyprogesterone caproate while the other half received an equivalent volume in an iden-

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Table I. Patient age and obstetric history

Group	*Age (yr)*	*Mature delivery (mean)*	*Premature delivery (mean)*	*Spontaneous miscarriages (mean)*	*Induced abortion (mean)*
Treated (n = 39)	27.8 ± 4.6*	1.5 ± 0.7*	1.4 ± 0.5*	2.5 ± 1.8*	1.8 ± 1.4†
Control (n = 40)	28.3 ± 5.2*	1.7 ± 0.7*	1.3 ± 0.5*	2.2 ± 1.1*	1.2 ± 0.4†

*Not significant.
†$p < 0.01$.

tical ampule containing a placebo (oily solution).* The experiment was designed as a double-blind study, i.e., neither the patients nor the medical staff knew which medication had been assigned to the individual patient.

According to our departmental protocol in patients with obstetric histories similar to those of our study population, cervical suture was performed in all women as soon as the fetal heart tone was audible by Doptone. The suture was removed at gestation week 37. Duration of pregnancy was determined on the basis of menstrual data, obstetric examination, and ultrasonography performed during the first trimester and between gestation weeks 18 and 24. The newborn infants were examined by a neonatologist immediately after delivery and 6 weeks later.

For the purposes of this study, miscarriage was defined as removal or expulsion from the uterus of an embryo prior to gestation week 20 or of an embryo weighing <500 gm or measuring <25 cm. Delivery was termed premature if it occurred at or before 36 weeks of gestation and if birth weight was ≤2500 gm. The criteria for imminent premature labor were (1) at least three regular uterine contractions within a 30-minute period registered by external tocography and (2) cervical effacement, but with dilation not exceeding 4 cm. Patients admitted to the hospital in imminent premature labor were treated, after removal of the cervical suture, with a combination of ritodrine and indomethacin according to our previously published protocol.[6]

Statistical significance was calculated by the proportion test.

Results

One patient from the treated group discontinued medication at 25 weeks of gestation and was dropped from the study because she moved away.

The two groups were found to be similar with regard to age and obstetric history (Table I). There was no statistically significant difference between the means, except for a larger number of induced abortions in the study group, but this does not seem to be relevant to the results obtained. Socioeconomic status, estimated by years of schooling and profession, was quite similar in the two groups, as was the ethnic origin of the patients. All the women were drawn from a relatively small geographic area. Three women of the treated group and two of the control group were smokers. Medication was started at a mean gestational age of 12.2 ± 3.3 weeks in the treated group and at 12.2 ± 3.9 weeks in the control group.

Eight (20.4%) patients from the treated group and three (7.5%) from the control group had miscarriages. The duration of treatment in these cases had ranged from 4 to 9 weeks and from 4 to 10 weeks, respectively; these intergroup differences were not statistically significant. The incidence of premature rupture of membranes occurring before 37 weeks of gestation (excluding miscarriages) was 6.4% (2/31) in the treated group and 8.1% (3/37) in the control group. These differences were not statistically significant (Table II).

Imminent premature labor occurred in a significantly smaller number of patients in the treated group (9/31, 29.0%) than in the control group (22/37, 59.4%) ($p < 0.025$). (In these and in the following results the miscarriages are excluded.) All of these women were treated with ritodrine and indomethacin. The rate of premature deliveries was also significantly lower in the treated group (5/31, 16.1%) than in the control group (14/37, 37.82%) ($p < 0.05$). The mean duration of pregnancy in the treated group was 38 ± 3.2 weeks for nonpremature cases and 32.4 ± 4.0 weeks for premature cases; the corresponding durations in the control group were not significantly different, i.e., 37 ± 3.7 and 33.8 ± 2.6 weeks, respectively.

There were no cases of perinatal death or fetal malformation in either of the groups. The mean birth weight of all infants from the treated group was significantly higher than that of the control group. However, when the premature infants in both groups were compared, their mean birth weight did not differ significantly. The same was true when all mature babies were compared (Table III).

There was no perinatal morbidity among the mature newborn infants in either of the groups. The difference in mean duration of morbidity among the premature infants of the treated group and of the control group

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*We are indebted to Taro Pharmaceutical Industries, Ltd., Haifa, Israel, for providing the 17α-hydroxyprogesterone caproate and the placebo for this double-blind study.

Table II. Outcome of current pregnancies

	Miscarriage	*Premature rupture of membranes (before 37 weeks)*	*Imminent premature labor*	*Premature delivery*
Treated group				
No. of patients with outcome	8*	2*	9†	5‡
% of patients with outcome	20.4	6.4	29.0	16.1
Total in group (n)	39	31	31	31
Control group				
No. of patients with outcome	3*	3*	22†	14‡
% of patients with outcome	7.5	8.1	59.4	37.8
Total in group (n)	40	37	37	37

*Not significant.
†$p < 0.025$.
‡$p < 0.05$.

Table III. Mean birth weight of infants in study

	Total deliveries	*Term delivery*	*Premature delivery*
Treated group			
Birth weight (gm)	3111.9 ± 905.5*	3406 ± 617.5†	1580 ± 518.4†
Range (gm)	810-4850	2700-4850	810-2080
No. of infants	31	26	5
Control group			
Birth weight (gm)	2680 ± 813.4*	3161.7 ± 484.3†	1888.6 ± 591.6†
Range (gm)	800-4540	2690-4540	800-2480
No. of infants	37	23	14

*$p < 0.05$.
†Not significant.

Table IV. Perinatal morbidity of premature infants

Main diagnosis	*Treated group (n = 5)*	*Control group (n = 14)*
Hyperbilirubinemia	4	11
Respiratory distress syndrome	1	4
Anemia	—	3
Apnea/bradycardia	—	2
Sepsis	1	2
Patent ductus arteriosus	1	—

was not significant (60.6 ± 57.9 days versus 50.1 ± 63.8 days). The total duration of hospitalization was 303 days for the five premature infants of the treated group and 702 days for the 14 premature infants of the control. The main reasons for hospitalization are summarized in Table IV.

Comment

The results of the present study confirm the findings of Johnson et al.[4, 5] that 17α-hydroxyprogesterone caproate is effective in preventing premature delivery. The mechanism of action of the drug is not yet known; however, its clinical usefulness was shown in our study by the reduction in the incidence of imminent premature labor from 59.4% in the control to 29.0% in the treated group and by a corresponding reduction in the incidence of premature delivery from 37.8% to 16.1%.

The differences between the two groups could not be explained in terms of obstetric history, time of commencement of treatment, or frequency of premature membrane rupture, since these were similar in both groups. The higher rate of miscarriages in the treated group does not seem to support the choice of this drug as a therapeutic agent for the prevention of spontaneous abortion, but the relatively late commencement of treatment with 17α-hydroxyprogesterone caproate (at 12.2 ± 3.3 weeks in the treated group and 12.2 ± 3.9 weeks in the control group) should be pointed out.

The mean birth weight and mean duration of pregnancy were not significantly different in either the mature or the premature subgroups; thus the significant difference between the treated and control groups with regard to the mean birth weight of all newborn infants derives from the difference in the number of premature infants born in the two groups. Although there was no perinatal death in either group, the incidence of morbidity for all premature infants was quite high. Treatment with 17α-hydroxyprogesterone caproate resulted in a reduction in the number of such cases, with an effective saving of some 400 days of hospitalization in the neonatal intensive care unit.

With regard to the cost/benefit ratio of treatment with 17α-hydroxyprogesterone caproate, the risk of the drug's teratogenicity toward the human fetus has still to be determined. We were encouraged by the fact that careful examination of the newborn infants in our study revealed no incidence of malformation. Although hydroxyprogesterone has not been found to be teratogenic in humans,[7, 8] some authors recommend extreme caution in the use of this medication.[5] The results of the present study demonstrate the immediate benefits of treatment with 17α-hydroxyprogesterone caproate. However, extended follow-up is needed in order to determine whether the drug exerts any long-term effects on the infant.

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Prevalence of papillomavirus infection in colposcopically directed cervical biopsy specimens in 1972 and 1982

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A total of 1264 consecutive cervical biopsy specimens obtained at the Parkland Memorial Hospital Dysplasia Clinic during 1972 were reviewed. Histopathologic specimens were assessed with special reference to changes induced by human papillomavirus. In 1972, only 0.7% of biopsy specimens were reported as consistent with human papillomavirus infection. Upon review, however, 36.5% of these specimens were found to demonstrate histologic criteria for the diagnosis of human papillomavirus infection. Approximately half of biopsy specimens reclassified as human papillomavirus were originally interpreted as inflammation; the others were interpreted as cervical intraepithelial neoplasia. Patients with human papillomavirus infection were significantly younger than patients with cervical intraepithelial neoplasia (24.9 versus 30.2 years). These findings were compared with 965 cervical biopsy specimens obtained in 1982. Thirty-four percent of these biopsy specimens revealed human papillomavirus infection. These observations support the concept that human papillomavirus infection of the cervix is not a new entity but a previously unrecognized finding whose prevalence has been relatively stable over a 10-year period. (AM J OBSTET GYNECOL 1985;151:577-81.)

Key words: Cervix, infection, papillomavirus, cervical intraepithelial neoplasia

Infectious agents have been implicated in the etiology of precancerous lesions of the cervix.[1, 2] Human papillomavirus, a subgroup of the papovavirus family, consists of a heterogeneous group of at least 16 types of viruses responsible for skin warts and condylomatous lesions of mucous membranes including that of the genital tract.[3, 4] Considered a rarity only a decade ago, human papillomavirus infection of the cervix is now

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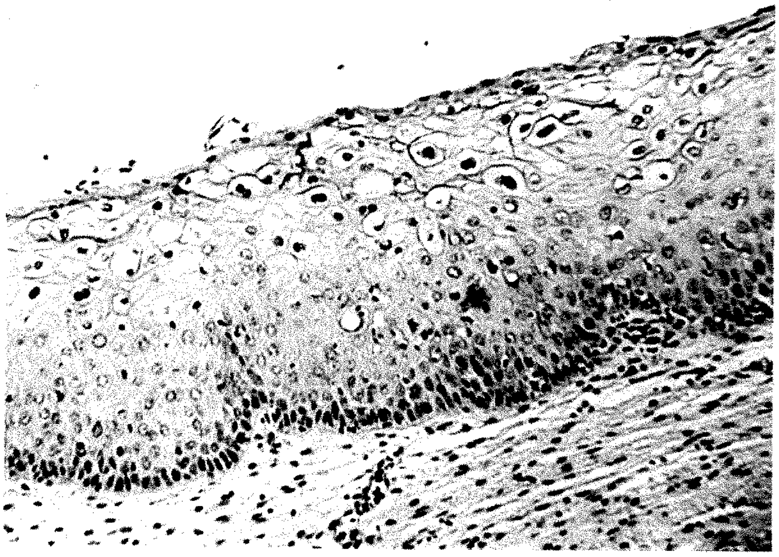

Fig. 1. Photomicrograph of a cervical biopsy specimen from 1972 showing the typical features of flat condyloma. There is a distinct basal layer with immediate maturation. There is no evidence of cervical intraepithelial neoplasia.

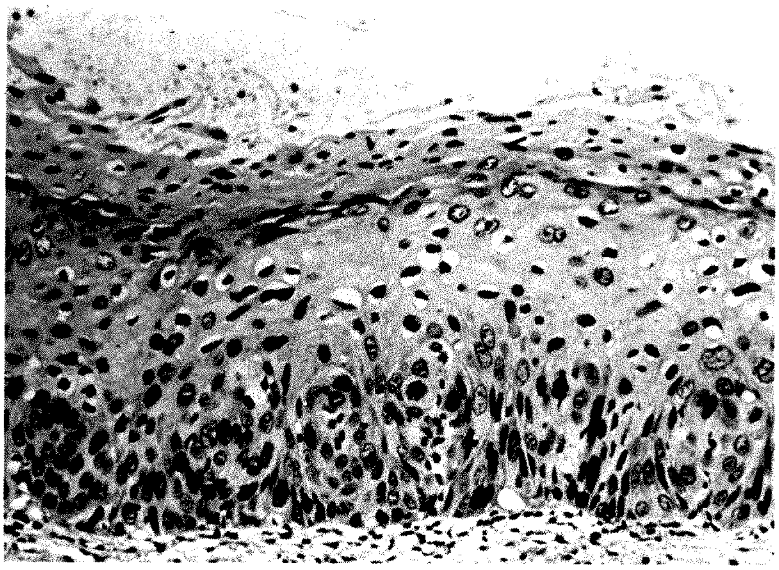

Fig. 2. Photomicrograph of a cervical biopsy showing features of both flat condyloma and grade 2 cervical intraepithelial neoplasia. Proliferation of neoplastic cells into the middle third of the epithelium is evident.

being diagnosed in 1% to 2% of women screened cytologically.[5]

Until 1976, condyloma acuminatum was known as a disease of the external genitalia and perianal region. The diagnosis was usually established clinically, and the pathologist was familiar only with the classical papillary growth. In 1976, Meisels et al.,[6] in a retrospective review, showed that 70% of lesions originally thought to be low-grade cervical intraepithelial neoplasia were attributable to the effects of human papillomavirus. These lesions are termed "flat condyloma" or "condyloma planum" because they lack the papillary configuration and are best seen with the colposcope. The purpose of this study was to review retrospectively all cervical biopsy specimens obtained in the dysplasia clinic at Parkland Memorial Hospital for a 12-month period both before and after the recognition of this entity and to assess the prevalence of human papillomavirus infection of the cervix over a 10-year period of time.

Table I. Distribution of histologic findings

	1972				*1982**	
	Original†		*Revised*			
Histologic finding	*No.*	*%*	*No.*	*%*	*No.*	*%*
HPV (sole finding)	9	0.7	461	36.5	328	34.0
CIN plus HPV						
Grade 1 CIN plus HPV	0	0.0	91	7.2	63	6.5
Grade 1 CIN plus HPV	0	0.0	51	4.0	48	5.0
Grade 3 CIN plus HPV	0	0.0	5	0.4	6	0.6
CIN (sole finding)						
Grade 1 CIN	253	20.0	22	1.7	17	1.8
Grade 2 CIN	227	18.0	26	2.1	23	2.4
Grade 3 CIN	126	10.0	29	2.3	37	3.8
Invasive cancer	26	2.1	26	2.1	12	1.2
Squamous metaplasia and/or inflammation	623	49.2	553	43.7	431	44.7
Total	1264	100.0	1264	100.0	965	100.0

HPV = Human papillomavirus; CIN = cervical intraepithelial neoplasia.
*Not significantly different from 1972 revised data ($p > 0.05$).
†Significantly different from 1972 revised data ($p < 0.001$).

Material and methods

A total of 1264 consecutive cervical biopsy specimens collected in 1972 and 965 consecutive cervical biopsy specimens collected in 1982 were reviewed by one of the authors (R. L. V.). All biopsies were performed in the dysplasia clinic. The patients consisted of an indigent clinic population referred because of an abnormal Papanicolaou smear. Colposcopy was performed after the application of 3% acetic acid to the cervix with the use of 15-fold magnification. Biopsy specimens were taken from areas representing the most severe changes. Specimens were fixed in 10% formalin, processed onto paraffin, and stained with hematoxylin and eosin.

The following criteria were used to identify human papillomavirus infection in histologic preparations: koilocytosis, dyskeratosis, parakeratosis, multinucleation, and spiked surfaces. The diagnosis of human papillomavirus infection (without cervical intraepithelial neoplasia) was formulated when the cellular architecture did not deviate from normal in the epithelial layers between the basement membrane and the human papillomavirus changes (Fig. 1). The diagnosis of human papillomavirus infection with cervical intraepithelial neoplasia was established when the features of neoplasia were observed in layers directly below or adjacent to human papillomavirus changes (Fig. 2). Histologic criteria for diagnosing cervical intraepithelial neoplasia included loss of basal/parabasal interface, upward polarity of nuclei, loss of maturation, mitotic activity, and abnormal mitoses.

Statistical analysis with the χ^2 test was used to compare differences in the distribution of histologic diagnoses between 1972 and 1982. Analysis of variance was used to compare mean ages between diagnostic categories in the 1972 and 1982 series.

Results

The histopathologic findings are summarized in Table I. In nine of 1264 (0.7%) cases of the original 1972 series the histologic diagnosis was condyloma acuminatum. A review of these charts revealed that these biopsy specimens were taken from clinically apparent papillary condyloma acuminatum. Forty-eight percent of biopsy specimens showed cervical intraepithelial neoplasia while 49.2% revealed benign changes of squamous metaplasia and/or inflammation. Review of these 1264 cases a decade later revealed that in 461 (36.5%) the sole finding was human papillomavirus infection. All but nine of these were flat condyloma. Cervical intraepithelial neoplasia as a sole finding was present in only 6.1% of cases. One hundred forty-seven (11.6%) cases demonstrated findings consistent with both human papillomavirus changes and cervical intraepithelial neoplasia. Analysis of the distribution of histologic findings showed a highly significant difference between the original and reviewed 1972 series ($p < 0.001$). Comparison of the 1972 reviewed series with 965 biopsy specimens from 1982 shows a remarkable similarity (Fig. 3). The χ^2 analysis reveals that the findings obtained in the 1972 review and the 1982 series were not significantly different ($\chi^2 = 11.07$, $p > 0.05$).

An analysis of 461 biopsy specimens from 1972 that were found to have the sole finding of human papillomavirus infection upon review is presented in Table II. The original 1972 diagnoses in these cases indicate that most condylomatous lesions were classified as ei-

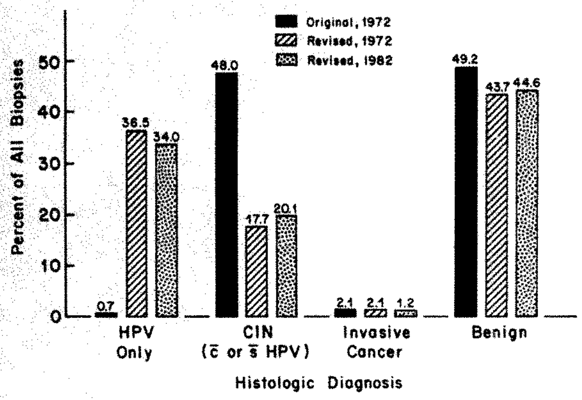

Fig. 3. Comparison of distribution of histologic diagnoses (original, 1972; revised, 1972 and 1982).

Table II. Original histologic diagnosis of biopsy specimens from 1972 that upon review showed only human papillomavirus infection

Original diagnosis	*No.*	*%*
Inflammation	233	51
Grade 1 CIN	187	41
Grade 2 CIN	32	7
Grade 3 CIN	9	2
Total	461	100

CIN = Cervical intraepithelial neoplasia.

ther low-grade cervical intraepithelial neoplasia or benign (inflammation and/or squamous metaplasia).

An analysis of patient age by diagnostic category is shown in Table III. In both 1972 and 1982, patients with human papillomavirus only were younger than those with human papillomavirus and cervical intraepithelial neoplasia, who in turn were younger than those with cervical intraepithelial neoplasia only ($p < 0.001$).

Comment

Cellular manifestations of human papillomavirus infection of the cervix were first observed in 1949 by Ayre.[7] In 1960, the same author[8] implicated a virus as the mechanism effecting these cellular abnormalities but the true significance of these findings was not recognized until recently. It has been reported that the histologic diagnosis of early cervical intraepithelial neoplasia was often incorrect and that in the majority of these cases the true diagnosis is a reflection of human papillomavirus infection of the cervix.[6, 9] Our review of 1264 biopsy specimens obtained in 1972 confirms these findings. Forty-eight percent of the specimens were originally classified as cervical intraepithelial neoplasia. With present-day criteria, as outlined in this report, only 17.7% of specimens demonstrated cervical intraepithelial neoplasia, either as a sole finding or in combination with human papillomavirus.

Table III. Mean age of patients, by histologic findings

Histologic diagnosis	*1972* *No.*	*1972* *Mean age** *(yr)*	*1982* *No.*	*1982* *Mean age†* *(yr)*
HPV only	461	24.9	328	22.8
HPV plus CIN	147	27.0	117	26.3
CIN only	77	30.2	77	29.0
Total	685	26.0	522	24.5

HPV = Human papillomavirus; CIN = cervical intraepithelial neoplasia.

*F_2,851 = 51.4; $p < 0.001$.

†F_2,521 = 64.7; $p < 0.001$.

The second striking finding concerns the diagnosis of human papillomavirus infection in patients referred with abnormal cervical cytologic tests. In 1972, only nine patients (0.7%) had biopsy specimens that contained the histologic finding of a condylomatous lesion. These were biopsy specimens of clinically apparent condyloma acuminatum. Upon review of the specimens an additional 452 biopsy specimens were added to the original nine cases, for a total of 461 (36.5%) cases of human papillomavirus infection.

Review of our data reveals that in 1972 11.6% of biopsy specimens contained both human papillomavirus and cervical intraepithelial neoplasia features. In 1982, 12.1% contained human papillomavirus and cervical intraepithelial neoplasia changes. Features of human papillomavirus infection were present in 66% in 1972 and 60% in 1982 of cases with cervical intraepithelial neoplasia (respectively), which compares favorably with the figures reported by Syrjanen[10] in which human papillomavirus infection was demonstrated in 50% of patients with cervical intraepithelial neoplasia.

The presence or absence of abnormal mitotic figures appears to be the most accurate criterion for distinguishing between cervical intraepithelial neoplasia (aneuploid) lesions and viral (diploid) lesions.[11, 12] This criterion was used in this report to categorize lesions into the following groups: human papillomavirus, cervical intraepithelial neoplasia with human papillomavirus, and cervical intraepithelial neoplasia.

In both the 1972 and 1982 series, women with the sole finding of human papillomavirus infection were younger than women with both human papillomavirus and cervical intraepithelial neoplasia who in turn were younger than those with the sole finding of cervical intraepithelial neoplasia. This concurs with Syrjanen's[13] finding that the mean age of women with condylomatous lesions of the cervix (28.5) was significantly lower than that of those with cervical intraepithelial neoplasia lesions (34).

This study also confirms others[6, 9, 14] that show human papillomavirus infection of the cervix to be a common finding in patients referred with an abnormal Papanicolaou smear. The lesions that result from the infection are flat rather than papillomatous. In many instances they are apparent only when viewed with a colposcope. This has contributed to the lack of recognition of human papillomavirus infection involving the cervix and rare reports in the literature prior to 1976.

From the data presented, we conclude: (1) that human papillomavirus infection of the cervix is a frequent lesion that often went unrecognized or was diagnosed as cervical intraepithelial neoplasia, (2) that human papillomavirus is commonly associated with cervical intraepithelial neoplasia, (3) that patients with human papillomavirus infection are younger than those with cervical intraepithelial neoplasia, and (4) that the prevalence of human papillomavirus infection of the cervix has been relatively stable over a 10-year period.

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A prospective study of herpes simplex virus infection in a defined population in Houston, Texas

Karen Adler-Storthz, Ph.D., Gordon R. Dreesman, Ph.D., Raymond H. Kaufman, M.D., Joseph L. Melnick, Ph.D., and Ervin Adam, M.D.
Houston, Texas

A seroepidemiologic study was conducted to determine the incidence of antibodies to herpes simplex virus in a population of middle-class Caucasian women. In utero exposure to diethylstilbestrol did not influence the frequency of herpes simplex virus infection. The frequency of infection increased with age and number of sex partners and a 50% increase in incidence of herpes simplex virus type 2 infection was noted during the 4-year period of the study. Subclinical herpes simplex virus type 2 infections apparently occurred in some women without preexisting antibodies to herpes simplex virus type 1. (AM J OBSTET GYNECOL 1985;151:582-6.)

Key words: Herpes simplex virus, infection, serology, epidemiology

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) cause a spectrum of clinical diseases. HSV-2 is responsible for one of the most common venereally transmitted infections and HSV-1 has been isolated from a large number of cases of genital HSV infection.[1-8] On the basis of seroepidemiologic studies, the incidence of HSV infection varies from population to population depending on age, race, socioeconomic status, and sexual practices. This study presents serologic data concerning the incidence of HSV infection in a population of middle-class Caucasian women as determined by a solid-phase microradioimmunoassay for detection of type-specific antibody to HSV. The specific aims of the study were: (1) to determine the prevalence of antibodies to HSV-1 and HSV-2 at the time of enrollment, (2) to study the development of antibodies to HSV-1 and HSV-2 during a follow-up period, and (3) to compare prospectively the presence of antibodies with history of oral and/or genital HSV infection.

Material and methods

Population studied. Women included in the study were participants in the diethylstilbestrol adenosis (DESAD) program in Houston, Texas who agreed to participate in a serologic study of HSV infections. There were three groups studied: (1) women who were invited to participate in the DESAD study after review of their mothers' records (record review group), (2) women who were referred by other physicians or who initiated their enrollment because they knew about their previous exposure to diethylstilbestrol (walk-ins and referrals), and (3) women who were matched to the record review group by age and by their mothers' ages and who were not exposed to diethylstilbestrol according to the same record source. The mean ages of the groups at enrollment were 22, 21, and 21 years, respectively. All women were middle-class Caucasians. Serum samples and a history of clinically manifest oral and/or genital infection with HSV were obtained during each visit.

Assay for antibodies to HSV-1 and HSV-2. Sera were stored at −20° C. The presence of type-specific anti-HSV antibodies was determined by a solid-phase microradioimmunoassay.[9, 10] The antigens used were the major glycoprotein antigens VP123 for HSV-1 and VP119 for HSV-2. All sera were tested at a 1:10 dilution and iodine 125–labeled goat IgG antihuman IgG was used as the probe.

Statistical analysis. The significance of differences between groups was determined by the χ^2 test. For cells numbered <6, Fisher's Exact Test was used.

Results

Table I presents data on the incidence of antibodies to HSV major glycoprotein antigens in both diethylstilbestrol-exposed and unexposed women. The enrollment serum sample was tested for each woman. Of the women exposed to diethylstilbestrol, the walk-in and referral groups had significantly more women (57%) with vaginal epithelial changes than did the rec-

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Table I. Antibodies to HSV by cohort

Antibodies to HSV	*Exposed to diethylstilbestrol*				*Unexposed, record reviews*	
	Walk-ins and referrals		*Record reviews*			
	n	%*	*n*	%*	*n*	%*
Type 1	16	30	33	38	29	33
Type 2	5	9	9	10	14	16
No. of women examined	54		88		88	

*Percent of column.

ord review group (10%, $p < 0.001$). Of the women exposed to diethylstilbestrol, those with vaginal epithelial changes did not differ significantly from those with no changes with respect to frequency of antibodies to HSV; therefore, these two categories were pooled in the table. There was no significant difference in the frequency of antibodies to HSV-1 or HSV-2 between the diethylstilbestrol-exposed groups. In the unexposed group, 33% of the women had antibodies to HSV-1 and 16% to HSV-2. These values were not significantly different from those of the diethylstilbestrol-exposed groups; therefore, all women were subsequently pooled for antibody studies in relation to age, history of HSV, and number of sex partners. When all women were pooled, 28 (12%) had antibodies to HSV-2 and 78 (34%) had antibodies to HSV-1.

The prevalence of antibodies to HSV by age is shown in Fig. 1. The percentage of women in the ≤19 age group with antibodies to HSV-1 was higher than, but not significantly different from, those in the other two groups. As expected, the frequency of antibodies to HSV-2 increased with age. There was a significant difference between HSV-2 antibody frequency between the ≤19 age group and the ≥23 age group (5% versus 17%, $p = 0.04$).

Fig. 2 presents the prevalence of antibodies to HSV by number of sex partners. While the percentage of women with antibodies to HSV-1 remained fairly constant as the number of sex partners increased, there was an increase in frequency of antibodies to HSV-2. The frequency of antibodies to HSV-2 in women with four or more sex partners was significantly higher than that of women who were virgins (3% versus 22%, $p = 0.02$) and that of women with one sex partner (8% versus 22%, $p = 0.04$). The presence of antibodies to HSV-2 in the one virgin was confirmed by microneutralization. Eighty-nine of 213 women (42%) had one or more new sex partners after 1 year in the study and 50 of the 89 (56%) had two or more (Table II). There was an increasing trend of incidence of antibodies to HSV-2 over time in all groups. The women with an increase of two or more sex partners had a significantly higher frequency of antibodies to HSV-2 in the second serum sample than those with no change in the number of sex partners (14% versus 30%, $p = 0.02$).

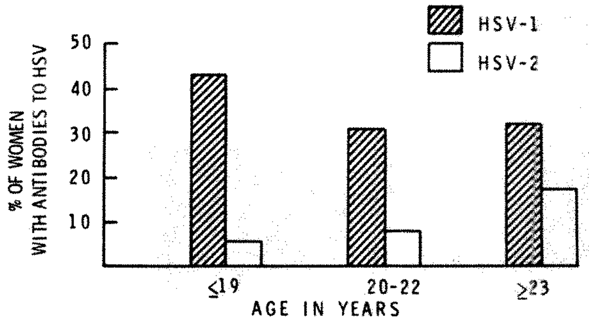

Fig. 1. Prevalence of antibodies to HSV by age.

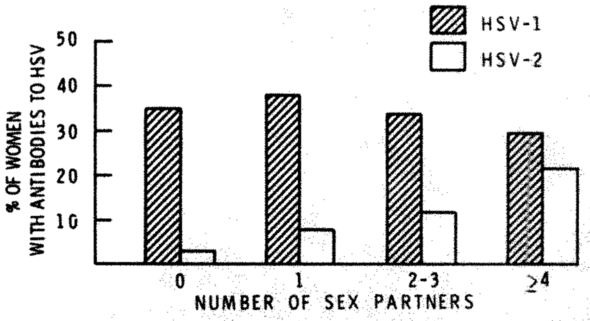

Fig. 2. Prevalence of antibodies to HSV by number of sex partners.

The data in Table III correlate the history of HSV infection with the prevalence of antibodies to HSV at the time of enrollment. Of the women with no HSV history (71%), 29% had antibodies to at least one of the HSV types and 8% had antibodies to HSV-2. Of 28 women with detectable antibodies to HSV-2, only eight (29%) reported a history of genital infection. Antibodies to HSV were not detected in 41% of the women who had a history of oral HSV infection.

Changes in history of HSV infection with time were correlated with antibodies to HSV in the follow-up specimen (Table IV). Twenty women reported a new history of HSV infection after ≥12 months in the study. Of the six with new oral infections, five had no change in antibody and one lost detectable antibody to HSV-1. Two of 12 women who reported new genital HSV infections had new antibody to HSV-2 only; one, to HSV-1 only; and one, to HSV-1 and HSV-2. One woman with previous antibodies to HSV-1 developed antibodies to HSV-2. Two women had antibody to HSV-2 in the first and second sera but reported genital infection only at the follow-up visit. The other five reporting new genital infections had no antibody to HSV in either serum sample. Two women reported new oral

Table II. Incidence of antibodies to HSV by increase in number of sex partners during the time period studied in 213 women

Increase in sex partners with time	*Serum sample**	*Antibodies to HSV*						*Total pairs of samples*
		HSV-1 only		*HSV-1 and HSV-2*		*HSV-2 only*	*None*	
		n	*%†*	*n*	*%‡*			
No increase	A	38	36	7	8	3	76	124
	B	34	34	8	14	9	73	
1	A	8	33	5	15	1	25	39
	B	9	38	6	21	2	22	
≥2	A	12	34	5	16	3	30	50
	B	11	42	10	30	5	24	

*A = Enrollment sample; B = follow-up sample ≥12 months after enrollment.

†Percent of all with antibodies to HSV-1 including those with antibodies to both types of HSV.

‡Percent of all with antibodies to HSV-2 including those with antibodies to both types of HSV.

Table III. Correlation of history with antibody prevalence

Antibodies	*History of clinical HSV infection*								*Total*	
	None		*Oral*		*Genital*		*Oral and genital*			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
HSV-1 only	34	21*	23	47	4	25			61	27
HSV-2 only	7	4			3	19	1	50	11	5
HSV-1 and HSV-2	7	4	6	12	4	25			17	7
None	115	71	20	41	5	31	1	50	141	61
Total	163	71†	49	21	16	7	2	1	230	

*Percent of column.

†Percent of row.

Table IV. Changes in antibodies to HSV during the time period studied

New history of HSV infection	*No. of women*	*Antibodies to HSV in enrollment sample*	*Antibodies to HSV in second sample*			
			HSV-1 only	*HSV-2 only*	*HSV-1 and HSV-2*	*None*
Oral	6	HSV-1 only	1	—	—	1
		HSV-2 only	—	1	—	—
		HSV-1 and HSV-2	—	—	1	—
		None	—	—	—	2
Genital	12	HSV-1 only	—	—	1	—
		HSV-2 only	—	2	—	—
		None	1	2	1	5
Oral and genital	2	None	—	—	—	2
None	19	HSV-1 only	—	—	4	—
		None	5	7	3	—

and genital infections but had no antibodies to HSV in either serum sample. Nineteen women did not report a new history of HSV infection but had serologic evidence of a new HSV infection; 15 of these did not have detectable antibodies to HSV in the enrollment serum sample but had newly detectable antibodies to HSV at the follow-up visit. Five had new antibodies to HSV-1 only; seven, to HSV-2 only; and three, to HSV-1 and HSV-2. Thus, 10 of these women apparently experienced a subclinical HSV-2 infection although they had no preexisting antibodies to HSV-1. Four women with preexisting antibodies to HSV-1 developed serologic evidence of subclinical HSV-2 infection.

Fig. 3 shows the incidence of HSV infection over a period of 4 years. It is apparent from the slope of the lines, determined by linear regression analysis, that during the second year of the observational period the incidence of HSV-2 infection increased by 6% while that of HSV-1 infection was unchanged. Thereafter the increase was similar.

Comment

This seroepidemiologic study has described the frequency of HSV infection in a well-defined population of middle-class Caucasian women. The frequency of HSV infection has been correlated with diethylstilbestrol exposure, age, number of sex partners, and clinical history.

Women exposed to diethylstilbestrol did not differ from the comparison group with respect to frequency of antibodies to HSV. The percentage of diethylstilbestrol-exposed women who develop vaginal epithelial changes reportedly varies from 34% to 65%.[11] When we compared those women with vaginal epithelial changes to those with no changes for the presence of antibodies to HSV, we found no significant differences in frequency of antibodies to either HSV type. Therefore, neither exposure to diethylstilbestrol per se nor the presence of vaginal epithelial changes appeared to influence the frequency of HSV infection in this population.

Our findings show that the frequency of HSV-2 infection increased with age while that of HSV-1 infection was fairly constant. Although the age groups in this study were different, other studies have reported similar age-related increases in the frequency of antibodies to HSV-2 as measured by the microneutralization test.[12, 13] Women with multiple sex partners were found to have a higher frequency of antibodies to HSV-2 than those with one sex partner or none. As the number of sex partners increased over time, so did the incidence of antibodies to HSV-2. This parallels the findings of other studies in that the frequency of genital HSV infection increases with increasing numbers of sex partners.[13, 14]

At the time of enrollment in the study, 12% of the women had antibodies to HSV-2. Vonka et al.[15] have recently reported a 15% frequency of antibodies to HSV-2 in a similar population of women. When the clinical history of HSV infection was correlated with the presence of antibody in the enrollment serum sample, 25% of the women reporting genital HSV infection had antibodies to HSV-1 only. This may be indicative of the increasing incidence of genital HSV-1 infections. Five women reporting genital infections were antibody-negative, thus raising the possibilities of an incorrect clinical diagnosis or of a primary infection that may have been missed by our assay.[10] Of these five, two women developed antibodies during the follow-up period, one to HSV-1 and one to HSV-2. This observation is compatible with the finding of late development of antibodies in some infected persons.

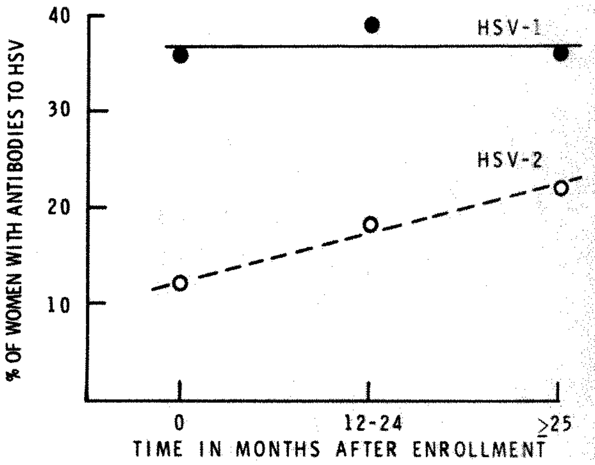

Fig. 3. Incidence of HSV infection by time.

Our findings suggest that subclinical genital infections with HSV-2 may occur even in the absence of preexisting antibodies to HSV-1. At the follow-up visit, 18 women had newly detectable antibodies to HSV-2; however, only four reported a new history of genital infection. Of the other 14, 10 did not have preexisting antibodies to HSV-1 but evidently experienced a subclinical infection.

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The effect of prostaglandin E_2 on the initial immune response to herpes simplex virus infection

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Numerous clinical situations, demonstrated to be associated with reactivation of herpes simplex virus infections, have also been shown to produce increases in local levels of prostaglandins. The current study was initiated to determine if prostaglandins play a role in the immune response and control of herpes simplex virus infections. Lymphocytes from volunteers were stimulated with concanavalin A, herpes simplex virus 1, or herpes simplex virus 2 in the presence of prostaglandin E_2 or ibuprofen, and the lymphocytes served as their own controls. The data suggest a statistically significant suppression in nonspecific T cell mitogen stimulation (concanavalin A), as well as specific herpes simplex virus 1 and 2 stimulations as measured by tritiated thymidine uptake by lymphocytes when stimulated in the presence of prostaglandin E_2. Ibuprofen did not alter the proliferative response to concanavalin A, herpes simplex virus 1, or herpes simplex virus 2 stimulation. This report examines the involvement of prostaglandins in herpesvirus infection such as the suppression of T cell function, allowing for a clinical recurrence. The usefulness of nonsteroidal antiinflammatory agents in the therapy of herpes simplex virus infections is also discussed. (AM J OBSTET GYNECOL 1985;151:586-90.)

Key words: Prostaglandin E_2, herpesvirus infection, immune response

The immune response to herpes simplex virus infections is complex and may differ in the primary and recurrent stages of the disease. Studies have suggested that the cell-mediated immune response is important in controlling and limiting herpes simplex virus.[1] A significant increase in recurrent disease with significant mortality and morbidity seems to occur when patients are treated with immunosuppresive agents.[2]

Within a clinical setting in an intact host, certain situations provoke reactivation of latent herpes. In women the most frequent correlation is with the onset of menstruation, but other situations such as fever, exposure to sunlight, emotional stress, and surgical resection have all been associated with an increased incidence of reactivation of latent herpes within the body.[3] All of these specific clinical situations have also been associated with increased local levels of prostaglandins. In exploring the role of prostaglandins in herpesvirus infection, it must be noted that these agents have been shown to increase viral multiplication and cell-to-cell spread of herpes simplex virus in specific in vitro models.[4, 5] Moreover, Blyth et al.[6] have produced a model in mice using herpes simplex virus 1 where reactivation of the virus was demonstrated after local inoculation of prostaglandin.

The specific study was undertaken to determine what role prostaglandins play in the immune response and

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control of latent herpesvirus infection. It found that localized prostaglandins may suppress the lymphocyte response to herpesvirus, thereby permitting recurrent disease within a localized anatomic site. By suppressing prostaglandin effects on lymphocytes, nonsteroidal antiinflammatory compounds may be useful in herpes simplex virus infection.

Material and methods

Patient population. A total of 61 patients were studied (56 females and 5 males) ranging in age from 18 to 45 years of age. Patients were referred from the community because of herpes infection or were recruited from the ambulatory service.

Three distinct patient populations were used for this specific study. Group 1 consisted of 22 normal healthy females, who had not taken any hormonal or oral contraceptive medicine for at least 6 months. They had no documented history of herpes simplex virus, either in the genital tract or oral cavity, and had a negative complement fixation titer to herpes simplex virus. Peripheral lymphocytes from these volunteers were stimulated with concanavalin A.

Group 2 included 24 patients (19 female, 5 male) with documented recurrent genital herpes simplex virus infections. These patients either had a positive herpes culture from the genital tract or cytologic and clinical evidence of genital herpes infection (positive complement fixation titer to herpes simplex virus). Their sensitized lymphocytes were stimulated with herpes simplex virus 2.

Group 3 included 15 patients (female) with documented recurrent oral herpes. They had a positive culture and serologic evidence of oral herpes. Sensitized lymphocytes were obtained from these patients and stimulated with herpes simplex virus 1. All patients signed informed consent forms, and the the study was approved by the institutional review committee.

Lymphocyte stimulation assay. Lymphocytes were separated from heparinized blood by Ficoll-Hypaque density centrifugation, washed twice in Hanks' balanced salt solution. Viable cell counts were performed using the trypan blue exclusion test, with cell counts adjusted to final concentration of 2 × 10^6 cells per milliliter in RPMI 1640 medium. Throughout the remainder of the experiment RPMI 1640 medium containing 2% calf serum with 2% D-glutamine and 1% penicillin and 1% streptomycin was used; 0.2 ml of cell suspension was seeded in flat-bottomed wells of a 96-well plate (Costar). After the appropriate concentration of nonspecific or specific mitogen was added, the microtiter plates were incubated in a humidified incubator containing 5% carbon dioxide. Cultures were stimulated with concanavalin A or herpes simplex virus 1, or 2 and incubated at 37° C for 48 hours. One microcurie of tritiated thymidine was added to each well, followed by an additional 24 hours of incubation time. Cells were harvested on filters and counted in toluene base scintillation fluid. Results are expressed as the stimulation index plus or minus the standard error of the mean. The stimulation index for concanavalin A was calculated as the mean counts per minute with concanavalin A divided by the mean in the unstimulated lymphocyte control wells. The stimulation index for herpes simplex virus antigens were calculated as the mean counts per minute from stimulated sensitized lymphocytes divided by the mean counts per minute with the lymphocyte cell control. Comparison between groups were calculated with use of the Wilcoxon signed-rank test.

Table I. Human peripheral lymphocytes from normal healthy adults (group 1) stimulated with concanavalin A and stimulated with concanavalin A in the presence of prostaglandin E or in the presence of ibuprofen (n = 22)

Concanavalin A	*Concanavalin A plus prostaglandin E*	*Concanavalin A plus ibuprofen*
9.9*	8.2	12.0
6.0	3.5	7.7
14.2	14.1	16.6
6.7	2.3	2.5
41.8	32.9	33.5
21.1	15.6	18.8
36.8	21.0	24.3
6.2	3.3	4.1
29.5	6.2	12.4
36.5	27.5	26.3
57.4	37.1	59.9
27.5	15.0	22.8
23.0	13.3	15.9
28.9	24.7	22.7
27.3	16.0	23.2
23.9	12.5	41.4
14.8	7.6	10.3
8.8	6.0	9.7
12.5	12.0	13.6
13.7	8.7	8.1
9.1	1.3	7.1
5.6	4.2	7.4
21.0†	13.3‡	18.2§
±2.9	±2.1	±2.9

*Stimulation index.

†Mean ± SEM.

‡$p < 0.005$ (Wilcoxon signed-rank test).

§Not significant.

Nonspecific and specific antigens. Concanavalin A, a nonspecific mitogen, was added in a final concentration of 1.5 μg/ml and was used to measure the proliferative phase of the T cell mediated immune response.

Confluent monolayers of human embryonal lung cells were infected with herpes simplex virus 1 (KOS strain) and herpes simples virus 2 (strain 186). When a 4+ cytopathic effect was observed, the supernatant

Table II. Human peripheral lymphocytes from patients with recurrent genital herpes (group 2) stimulated with herpes simplex virus 2 and stimulated with herpes simplex virus 2 in the presence of prostaglandin E or in the presence of ibuprofen (n = 24)

Herpes simplex virus 2	*Herpes simplex virus 2 plus prostaglandin E*	*Herpes simplex virus 2 plus ibuprofen*
3.8*	2.3	2.8
3.9	1.6	3.9
4.4	2.6	8.6
4.2	3.9	5.9
3.2	2.4	4.6
6.1	3.6	5.2
2.9	1.5	2.5
10.3	8.7	15.0
8.1	5.3	7.9
6.2	3.7	8.8
13.9	1.7	3.3
3.4	1.6	2.5
7.4	1.3	3.1
5.3	2.7	4.4
12.3	4.8	11.2
40.4	12.6	20.4
7.9	5.1	7.8
11.8	8.0	13.3
7.4	6.5	8.0
16.3	11.1	18.2
6.4	3.8	7.2
19.3	12.9	18.8
14.1	11.1	13.9
15.3	11.3	17.2
9.4† ±1.7	5.4‡ ±0.8	8.9§ ±1.2

*Stimulation index.
†Mean ± SEM.
‡$p < 0.005$ (Wilcoxon sign-rank test).
§Not significant.

Table III. Human peripheral lymphocytes from patients with recurrent oral herpes (group 3) stimulated with herpes simplex virus 1 and stimulated with herpes simplex virus 1 in the presence of prostaglandin E or in the presence of ibuprofen (n = 15)

Herpes simplex virus 1	*Herpes simplex virus 1 plus prostaglandin E*	*Herpes simplex virus 1 plus ibuprofen*
12.8*	6.0	7.3
17.1	10.4	15.8
3.0	1.6	5.3
25.1	14.4	30.7
11.5	9.5	16.8
15.2	10.4	14.4
6.9	4.4	7.1
15.7	10.4	14.9
31.3	17.7	27.1
21.5	13.1	22.9
4.5	2.5	10.3
1.7	1.4	2.7
13.8	8.8	14.9
13.8	6.5	15.1
28.1	18.8	28.8
14.1† ±2.1	9.1‡ ±1.4	15.6§ ±2.2

*Stimulation index.
†Mean ± SEM.
‡$p < 0.005$ (Wilcoxon sign-rank test).
§Not significant.

fluid and cells were harvested and the suspension was frozen and thawed three times. It was then centrifuged at 2800 rpm for 30 minutes, and aliquots were frozen at −70° C. Viruses were titered in Vero cells and had a concentration of 3.3×10^8 pfu/ml for herpes simplex virus 1 and 1.5×10^8 pfu/ml for herpes simplex virus 2. Control antigens were treated identically with the exception of the inoculation of virus. Virus was grown in human embryo lung cells to prevent any nonspecific stimulation of human peripheral lymphocytes by any foreign antigens that could be found in monkey cells (Vero). Herpes simplex virus 1 and 2 antigens as well as control antigens were treated at 56° C for 2 hours prior to use and were tested for infectivity prior to heat treatment and post inactivation. A total volume of 0.05 ml of antigen was added to each well. Assays were run in sextuplicate. All assays were conducted with the same pool of concanavalin A, herpes simplex virus 1, or herpes simplex virus 2.

Prostaglandin and ibuprofen. Prostaglandin E_2 and ibuprofen were added to the cells after the final wash. Prostaglandin E was added at a final concentration of 1 μg/ml while ibuprofen was used at a final concentration of 50 μg/ml. The compounds already in solution were supplied by Upjohn Company, Kalamazoo, Michigan.

Results

Table I presents results of human peripheral lymphocytes stimulated with concanavalin A from 22 patients who were normal, healthy adults (group 1). These lymphocytes stimulated with concanavalin A showed a stimulation index of 21.0 ± 2.9. When these same lymphocytes were stimulated with concanavalin A in the presence of prostaglandin E, there was a statistically significant reduction in tritiated thymidine uptake, with a stimulation index of 13.3 ± 2.1. This 37% reduction in stimulation index is statistically significant by means of the Wilcoxon signed-rank test with $p < 0.005$. In lymphocytes stimulated with concanavalin A in the presence of ibuprofen, there was no statistically significant increase or decrease in tritiated thymidine uptake. A mean stimulation index of 18.2 ± 2.9 was found in these cells stimulated with concanavalin A in the presence of ibuprofen.

Table II presents the data from 24 patients with recurrent genital herpes (group 2) whose peripheral lymphocytes were stimulated with herpes simplex virus 2. These sensitized lymphocytes stimulated with herpes simplex virus 2 had a mean stimulation index of 9.4 ± 1.7. When these same lymphocytes were stimulated with herpes simplex virus 2 in the presence of prostaglandin E, there was a 43% decrease in tritiated thymidine uptake, which was statistically significant ($p < 0.005$, Wilcoxon signed-rank test). Mean stimulation index was 5.4 ± 0.8. When these cells were stimulated with herpes simplex virus 2 in the presence of ibuprofen, the mean stimulation index was 8.9 ± 1.2. Not a statistically significant increase or decrease in tritiated thymidine uptake.

Table III presents the data from 15 patients with recurrent labial herpes (group 3) whose peripheral lymphocytes were stimulated with herpes simplex virus 1. These sensitized lymphocytes stimulated with herpes simplex virus 1 had a mean stimulation index of 14.1 ± 2.1. When these same lymphocytes were stimulated in the presence of prostaglandin E, there was a 35% reduction in tritiated thymidine incorporation, 9.1 ± 1.4 ($p < 0.005$, Wilcoxon signed-rank test). When these lymphocytes were stimulated with herpes simplex virus 1 in the presence of ibuprofen, there was no statistically significant decrease or increase in tritiated thymidine incorporation, with a mean stimulation index of 15.6 ± 2.2.

Patients without a prior history of herpes simplex virus and a negative complement fixation titer (<1:8) had stimulation indexes of <1.5 to herpes simplex virus 1 or 2, as demonstrated in these experiments.

Comment

The results of the present study indicate that prostaglandin E may have a function in the initial immune response in T cell dependent infections. The results also suggest that prostaglandin E may alter lymphocyte function and play a role in herpes simplex virus infection and reactivation. According to these data, ibuprofen apparently did not alter the initial proliferative immune response to a nonspecific T cell mitogen or to herpes simplex virus 1 or 2.

Elevated local levels of prostaglandins may change the immune balance between herpes simplex virus and the host and therefore lead to a herpesvirus recurrence.[7] Numerous investigators have reported suppression of human peripheral lymphocytes by prostaglandins. Smith et al.[8] demonstrated significant inhibition of tritiated thymidine incorporation into phytohemagglutinin-stimulated human lymphocytes by several prostaglandins. Low doses of prostaglandins of the E series were found to suppress mitogen stimulation of human lymphocytes,[9] and prostaglandins of the E series reportedly have been produced in mitogen stimulation of human leukocytes.[10]

Data have been accumulating that indicate that prostaglandins of the E series act as modulators in the human immune system.[9] The data presented in the current study confirm these findings with nonspecific mitogen stimulation and reveal the role of prostaglandin E in the specific proliferative immune response to herpes simplex virus 1 and 2. The current report supports the contention that prostaglandins are involved in the immune response to herpes simplex virus infection and that alterations in prostaglandin levels may lead to suppression of lymphocyte function, thus allowing localized recurrent disease. This is the first report of prostaglandin suppression of specific proliferative immune response to herpes simplex virus.

Some reports propose the possibility that prostaglandins produce a balance of the immune response within the host and that the endogenous synthesis of prostaglandins is involved in the regulation of lymphocyte activation.[11] Apparently, prostaglandin E activates a glass-adhered T suppressor cell, which in turn suppresses response of the nonadherent lymphocytes to mitogen.

There have also been reports suggesting that nonsteroidal antiinflammatory compounds enhance nonspecific mitogen stimulation.[9, 12] In this study, however, no significant stimulation of blastogenesis by ibuprofen, either with the nonspecific mitogen concanavalin A or herpes simplex virus 1 or 2, was found, agreeing with the finding of Novogrodsky et al.,[13] which showed no enhancement of the proliferative immune response with nonspecific mitogens with the use of the antiprostaglandin indomethacin.

In conclusion, although the role of prostaglandins in herpes simplex virus infections obviously requires further elucidation, at present it appears that prostaglandins may be directly involved in enhancing herpesvirus infection by allowing cell-to-cell spread. They may also cause indirect effects by lowering the host's immune response and suppressing the lymphocytes necessary to control the infection. The data from this investigation strongly suggest that the initial proliferative immune response is inhibited by prostaglandin E, and this finding clarifies some of the clinical settings in which reactivation of herpes occurs with elevated local prostaglandin levels, though it has not been examined whether prostaglandins are factors in other immune functions in herpes simplex virus infection. Some reports propose that prostaglandins depress macrophage and inhibitory factor activity,[14] leukocyte inhibitory factor production,[15] direct cytolysis by activated lymphocytes,[16] and some components of antibody production.[17] Whether antiinflammatory nonsteroidal compounds will be useful in herpesvirus infection or other infections requires additional experimental evidence.

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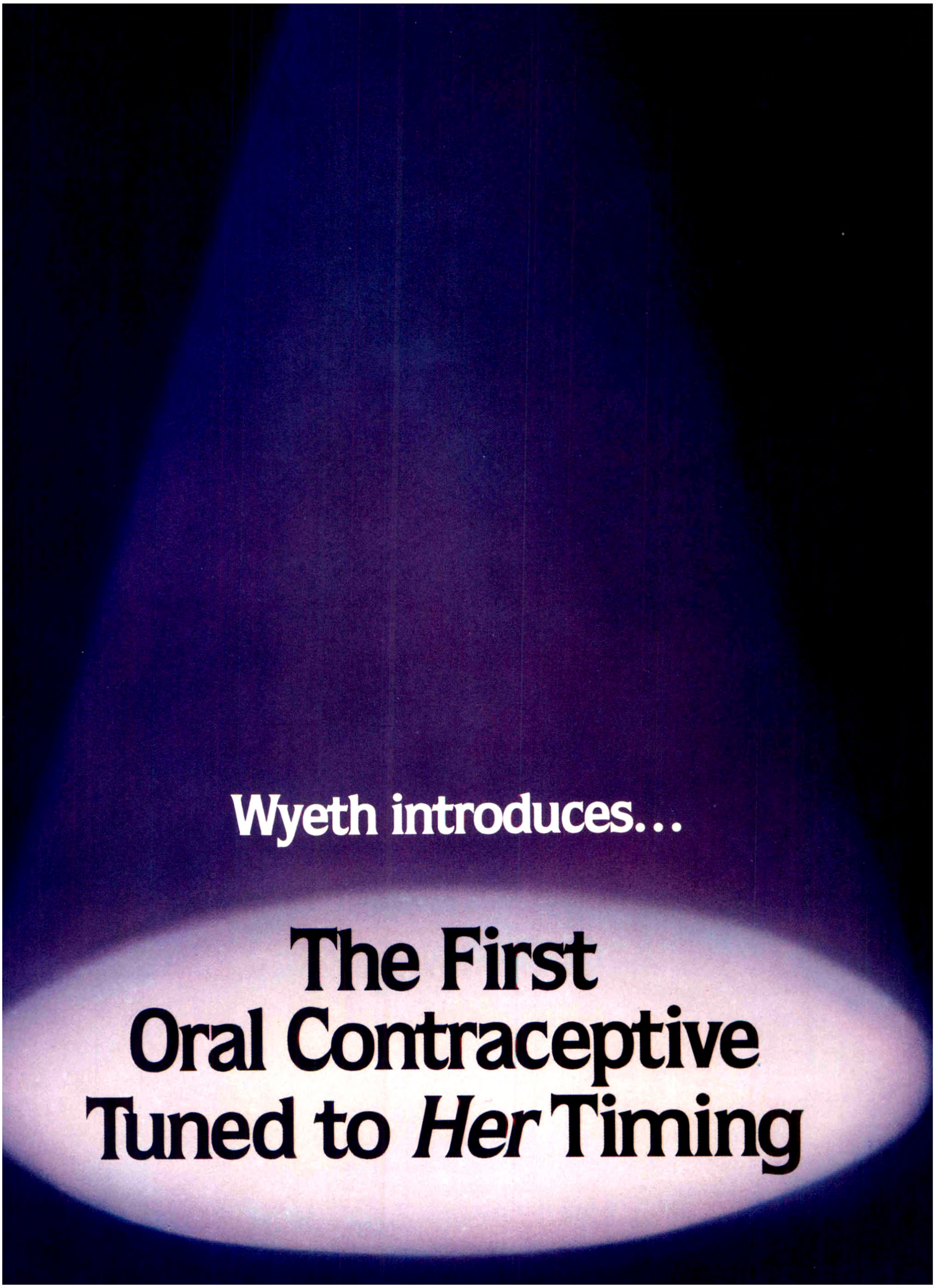
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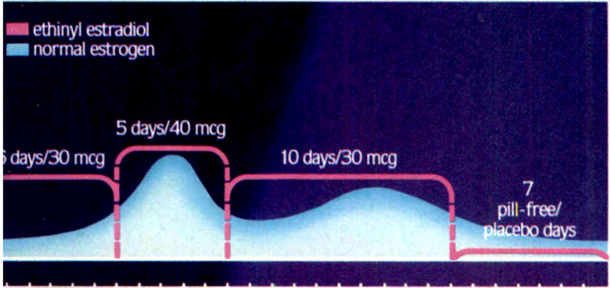

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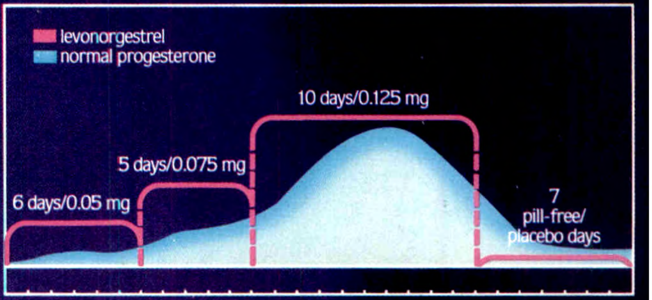

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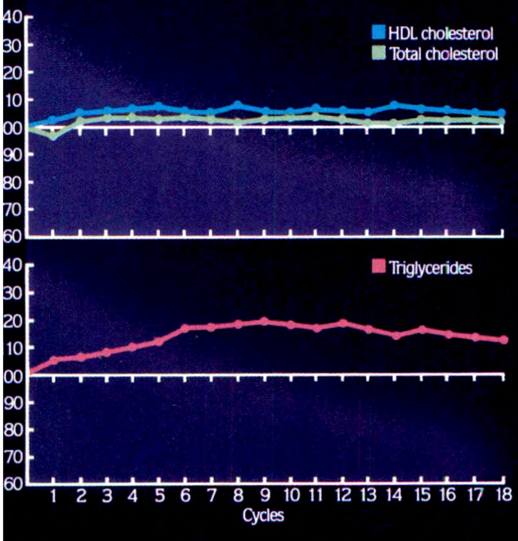
o significant impact n lipid levels shown in - and 18-month studies

vo separate 6-month studies demonstrate inimal impact on lipid levels; no statistically gnificant changes were observed in HDL, L, total cholesterol or triglycerides.[1,2] This further supported by a separate 18-month ospective study in which 85 non-smoking omen under the age of 35 were equally vided among 4 oral contraceptive formulans. With Triphasil, no statistically significant anges in total or HDL cholesterol occurred.[3] this study, slight increases in triglycerides, ich peaked at cycle 9, declined in subseent cycles.



ure 1: Monthly HDL cholesterol, triglycerides and total lesterol levels for Triphasil over an 18-month period.

No significant impact on blood pressure means shown in 24-month study

In clinical trials over 2 years, Triphasil had no significant impact on blood pressure means. As shown in Figure 2, a high degree of stability was maintained with Triphasil compared to baseline.[2]

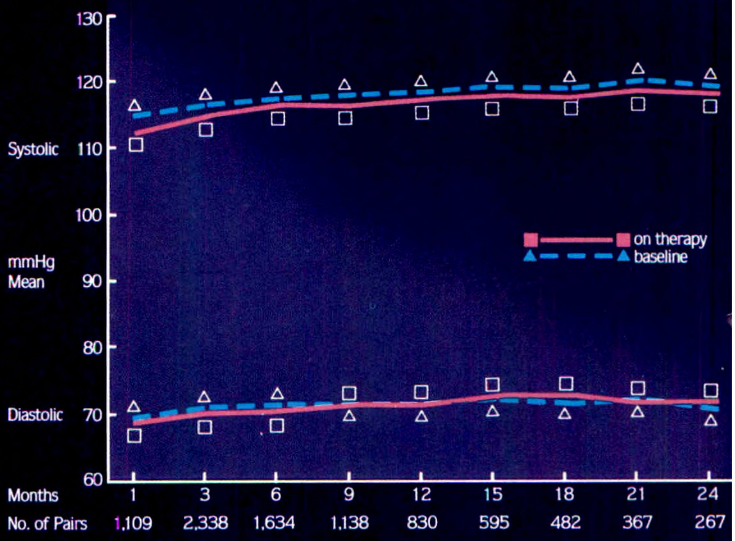


Figure 2: Blood pressure mean levels during treatment and baseline–systolic and diastolic.

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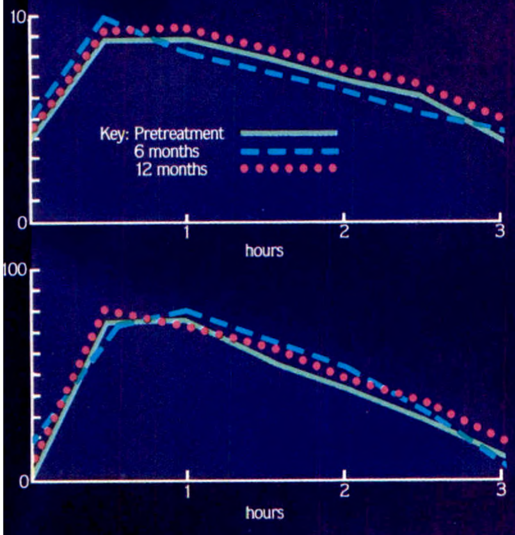
lo significant impact on arbohydrate metabolism n 18-month study

esults at 6, 12, and 18 months demonstrate iphasil has little influence on carbohydrate etabolism. Glucose tolerance and insulin lev- s generally remain within normal clinical mits. Figure 3 shows glucose tolerance test esults from a study of 85 non-smoking omen under the age of 35 who were equally vided among 4 oral contraceptive formu- tions. Blood samples were taken before iphasil and at treatment cycles 6, 12, and 18. indings at 18 months were not plotted ecause they were comparable to 12-month ata.[3]



ure 3: Oral glucose tolerance test results: Pretreatment and ring treatment cycles 6 and 12.

And a low level of minor side effects—including breakthrough bleeding*

The more natural phasing of *both* estrogen and progestogen in new Triphasil provides oral contraception that is characterized by a low incidence of breakthrough bleeding and minimal complaints of other minor side effects. This is demonstrated in extensive multicenter clinical trials spanning more than three years.[2]

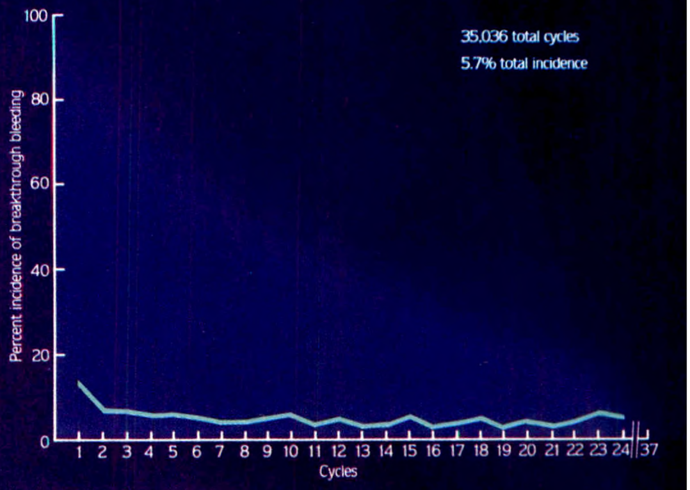


Figure 4: Incidence of breakthrough bleeding in 3,546 women during 24 months of Triphasil therapy.

New Triphasil®

Levonorgestrel and ethinyl estradiol tablets— Triphasic regimen

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21- and 28-day regimens

Worldwide experience confirms clinical studies

phasic OC that
eed for "Back-Up"
contraception
uring the first cycle
when used as directed
THU
FRI
SAT
SUN
MON
TUE
WED

starts from dose 1, *Her* day 1

Unique Dosage Regimen: Starts on *Her* Day 1

New Triphasil gives oral contraception a simple new start—*dose 1 begins on her day 1*. For the *first* cycle she'll take the *first* tablet on her *first* day of bleeding—eliminating the confusion of counting days or waiting for Sunday. It's easy for her to remember… and for you to explain.

No Need for "Back-Up" Contraception During the First Cycle When Used as Directed

Unlike oral contraceptives which utilize a Sunday-start regimen, with Triphasil no additional protection was used during clinical studies in 3,546 women, and no pregnancies occurred during the first cycle. In worldwide post-marketing experience, there has been only one first cycle pregnancy reported in the literature.[4] For dosage and administration, please refer to brief summary.

Day 1 Start— In Tune with *Her* Timing

Day 1 start means she starts on a day established by HER OWN biology, not by a calendar or package.

Elegant Compact—Discreet, Functional and Reusable

To keep it simple, New Triphasil has a new package—a discreet, easy-to-follow reusable compact she'll feel comfortable with from day 1.

New Triphasil®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

Wyeth™

21- and 28-day regimens

tuned to her timing

References: 1. Vermeulen A, Thiery M: Metabolic effects of the triphasic oral contraceptive Trigynon® [Triphasil®]. *Contraception 26*:505-513, 1982. 2. Data on file, Wyeth Laboratories. 3. Briggs MH in Brosens I, ed: *New Considerations in Oral Contraception*. Proceedings of an international

IN BRIEF:

TRIPHASIL®—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few day Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live birth the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no ev from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibl types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increas spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended tha any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If t patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becc pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clini recommend 3 months, although no precise information is available on which to base this. The administration progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of O and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significan percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three stuc were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some wome hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women w hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previousl had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hyper sion that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurr persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patien discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional caus should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time c change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potenti useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young wor without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinui OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use oth methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity an quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depres recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with care monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorc migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administ with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a wo becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency a complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing C

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measure protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, refle the elevated TBG: free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated w concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phen sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemo rhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an ass ciation between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nau and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patie during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuanc treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secr tion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminu in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomy omata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reporte users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, catarac changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Radiation treatment of carcinoma of the vulva, 1950-1980

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A retrospective study of all patients with carcinoma of the vulva treated by radiation therapy at the A. Maxwell Evans Clinic of the Cancer Control Agency of British Columbia, between 1950 and 1980, is reported. Sixty-eight patients, representing 30% of all referred patients with vulvar cancer, were analyzed for survival, recurrence patterns, complications, and clinical features. This group is companion to a series of patients treated with operation reported in 1979. The data confirm a major role for radiation therapy both in palliation and in combined radiotherapy-operation. For 13 cases, radiotherapy with curative intent was used in combination with operation resulting in a 5-year actuarial survival of 92%, with acceptable posttreatment morbidity. The advantages of preoperative radiotherapy, particularly for posterior vulvar lesions, are suggested by the data, and the need for a reappraisal of the role of radiotherapy in vulvar carcinoma is stressed. (AM J OBSTET GYNECOL 1985;151:591-7.)

Key words: Radiation, carcinoma, vulva

In 1979, the Gynecology Tumour Group of the Cancer Control Agency of British Columbia published its experience with treatment of carcinoma of the vulva from 1938 to 1976.[1] From that study of 264 patients has emerged this evaluation of the role of radiotherapy in the management of this disease. In particular, the use of limited volume radiation therapy combined with operation in selected patients has suggested that it is possible to reduce treatment-related morbidity yet achieve equal or better survival. Such patients include those with large cancers that are judged to be initially inoperable and those with lesions involving the fourchette, perineum, and anus. In this latter group curative surgical procedures involve morbidity even more extensive than that of radical vulvectomy since posterior exenteration is also necessary.

Methods and material

For the purposes of this study, charts of all patients who received radiation treatment for carcinoma of the vulva at the A. Maxwell Evans Clinic between 1950 and 1980 were reviewed. The year 1950 was selected as the initial date of evaluation since the first megavoltage equipment (cobalt 60) was introduced at this center about that time. A comprehensive computerized data sheet was completed for each patient. Survival data were prepared by means of a format based on the Kaplan-Meier method of analysis. Cross tabulations for occurrence frequencies were obtained by computer analysis.

A total of 230 cases of invasive vulvar carcinoma were registered at the A. Maxwell Evans Clinic in the years under consideration. Sixty-eight (30%) patients received either palliative or radical (curative intent) radiation therapy at some time in the course of the illness (squamous carcinoma, 64; malignant hidradenoma, one; Bartholin gland adenocarcinoma, three). Five radiotherapy treatment subgroups were identified: group 1, a group receiving palliative radiotherapy solely with the intent of relieving symptoms (40 cases); group 2, a group with recurrence after operation wherein radiation therapy was given with the intent of cure when disease had recurred more than 3 months after an attempt at curative operation (nine cases); group 3, a curative radiotherapy group consisting of patients treated by radiation alone with the intent of cure (six cases); group 4, a postoperative combined-treatment group wherein curative intent radiotherapy was given less than 3 months following operation (six cases); and group 5, a preoperative radiotherapy group (seven cases) (see Table II).

An analysis of recurrence was performed, which included the time from treatment to first recurrence as well as the site. An analysis of complications was performed in detail, and a rating scale of mild, moderate, or severe complications was used. Mild complications were defined as those that resulted in only minor signs and symptoms. Moderate complications were those that subjectively produced sufficient symptoms to require some treatment for control of pain or disability. Severe

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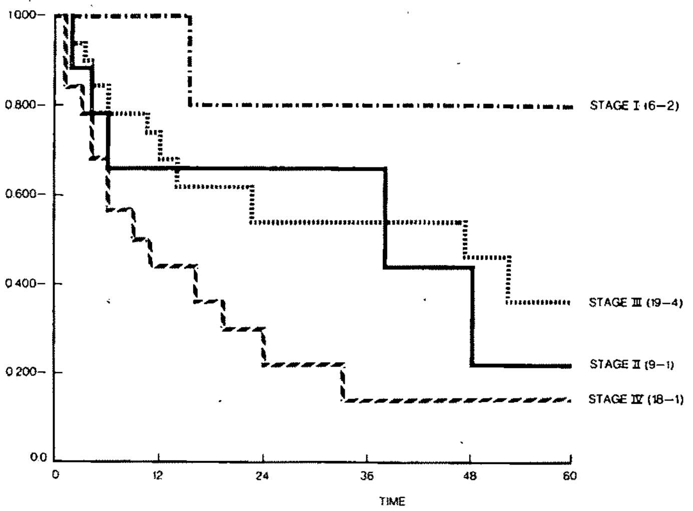

Fig. 1. Actuarial survival by TNM stage grouping.

Table I. Carcinoma of the vulva: Radiotherapy

Years	*No. of cases*
1950-1959	15
1960-1969	18
1970-1980	35
Total	68

complications were those that resulted in a significant alteration in the quality of life of the patient and/or required long-term use of medication for control of such symptoms.

The radiotherapy methods used over this time period varied considerably. The most common method of palliative irradiation therapy, either to the primary malignancy on the vulva or to metastatic nodes in the groin, was the application of cobalt 60 to a single megavoltage field with an appropriate bolus when indicated. When only short-term survival was expected, palliative doses of 3000 cGy in 10 fractions over 2 weeks were used. For patients with a longer expectation of survival, doses up to 5000 to 5500 cGy during 5 to 5½ weeks were given.

In group 3 patients (curative radiation therapy only), four of six patients were treated by radium needle implants only. This was common in the earlier years of the study and was not used in later years.

A variety of megavoltage treatment methods were used when radiation therapy was given after operation (group 4). The surgical treatment group included three patients with radical vulvectomy combined with bilateral inguinal-femoral lymphadenectomy, two patients with radical vulvectomy only, and one with partial vulvectomy. Three patients received radiotherapy to the vulvar and inguinal areas. This consisted of a 90-degree wedge pair, 8 cm wide by 10 cm long, arranged over the vulva and perineum. Where possible, the inferior extent of the perineal field came to the anal verge and the anterior field covered the mons pubis. A central contour of the vulva was obtained and the dose was prescribed at the intersection of the central axes of each field. Single anterior cobalt 60 fields were matched to the anterior vulvar field and were of sufficient size to encompass the inguinal surgical wound and inguinal-femoral node sites. A wax bolus was used over the lateral surgical scars. The central dose specified for the vulva was 5000 cGy during 5 weeks with the option to continue a further 500 cGy in two fractions if the vulvar skin reaction was not too severe by the end of the prescribed dose. Each inguinal field received a given (surface) dose of 5000 cGy during 5 weeks.

One patient received vulvar radiation by the wedge pair only, one received radiation by a single direct field to the vulva only, and one received only inguinal field radiation.

Beginning in 1970, planned preoperative radiation therapy (group 5) was initiated as a treatment policy in two clinical situations. In the first group, patients with large primary lesions thought to be inoperable received local radiotherapy in an attempt to render operation feasible. In the second group, patients with lesions encroaching on the perineal skin or anus received radiotherapy with the purpose of avoiding a posterior exenteration procedure, which would otherwise be required in combination with radical vulvectomy and lymphadenectomy.

In five of seven cases, the technique of radiotherapy involved a single direct megavoltage field with an appropriate bolus directed to the primary lesion and a margin of only 2 cm allowed only around visible or palpable disease. It is emphasized that no attempt was made to encompass the "at risk" volume (vulva and

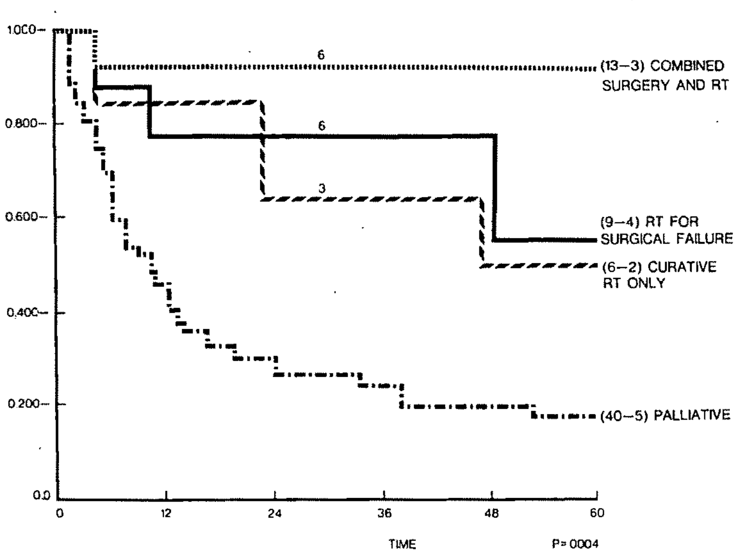

Fig. 2. Actuarial survival by treatment groups.

regional nodes) by high-dose radiation in order to minimize normal tissue complications from the combined operation-radiotherapy approach. A given (surface) dose of 5000 cGy was delivered during 5 weeks with a boost dose of 500 cGy in two fractions given at the end, with a reduction in field size if possible. One patient was treated by a radium needle implant and one by a wedge pair technique as previously described.

After the completion of radiotherapy, the planned operation was usually performed 6 weeks later in order to allow healing of any acute localized reaction and further regression of tumor size.

Results

Table I summarizes the distribution of cases by decade and Table II presents data for each treatment group. Staging refers to the stage groupings of the TNM classification determined clinically when diagnosis and treatment were first done.

Survival results. The curves for survival by stage (Fig. 1) include the influence of treatment for palliation only; in four of nine Stage II cases and eight of 19 Stage III cases treatment was considered as palliative. No patient with Stage I disease was treated palliatively, whereas 16 of 18 (89%) of patients with Stage IV were. Although obviously influenced by small numbers, the 5-year results (actuarial) were: Stage I, 80%; Stage II, 22%; Stage III, 37%; and Stage IV, 15%. The apparent difference in survival between Stage II and Stage III is influenced by the small numbers of patients and the results are further confounded by the long survival of some patients treated for palliation only. Furthermore, extensive T2 lesions (Stage II) may in fact be less curable than small T3 lesions (Stage III).

Fig. 2 presents survival by treatment intent and includes all cases. In those cases treated by combination operation and radiation (planned combined treatment), the 5-year actuarial survival was 92%. In cases with treatment for recurrence following operation, the 5-year survival was 56%, which is similar to our results with radical vulvectomy and inguinal femoral node dissection when the lymph nodes were found to be positive.[1] Curative radiotherapy alone resulted in an actuarial survival of 50% for six cases; however, four of these patients died of intercurrent disease with no evidence of local recurrence. The 5-year survival of the group treated for palliation was 17% with five of 40 patients surviving to 5 years.

Local control. Table III presents the data for curative-intent radiotherapy. Sixteen of 19 patients (84%) had local control of disease and local control was not achieved in only one patient in each of the preoperative and postoperative groups.

Nodal status and outcome. An analysis of the nodal status of patients in the group with curative radiotherapy only and the postoperative radiotherapy group is presented in Table IV. Of the radiotherapy only group, one patient (T3 N2) died of disease, four died of intercurrent disease, and one (T3 N1) is alive and well. In the group receiving radiotherapy after operation, all patients are alive, although one patient (TX NX) in whom the nodes were not removed at operation has recurrent disease. Of the remaining five who are well, three had ipsilateral nodes with disease and one had bilateral nodes with disease.

None of the seven patients receiving preoperative radiotherapy had clinically positive nodes. Four (T1, one; T3, two; T4, one) had negative nodes following definitive surgical procedures. Of three others (all T2), two received further local inguinal field radiotherapy

Table II. Carcinoma of the vulva: Radiotherapy, A. Maxwell Evans Clinic, 1950-1980

		*ECOG performance status**					*Histologic grade*			
Treatment group	*No. of patients*	*0*	*1*	*2*	*3*	*4*	*Well differentiated*	*Moderately differentiated*	*Poorly differentiated*	*Ungraded*
1. Palliative	40	21	9	2	4	4	11	14	10	5
					(25%)					
2. Recurrence after operation	9	8	1	—	—	—	2	1	4	2
3. Curative radiotherapy only	6	3	2	1	—	—	2	2	2	—
4. Operation and radiotherapy	6	4	2	—	—	—	4	2	—	—
5. Preoperative radiotherapy	7	3	4	—	—	—	4	—	3	—
Total No.	68	39	18	3	4	4	23	19	19	7
%							34		28	

*ECOG = Eastern Cooperative Oncology group (see reference 2).
†Did not complete therapy.

Table III. Treatment outcome

Group	*No.*	*Alive and well*	*Died of intercurrent disease*	*Alive with disease*	*Died of disease*
"Curative" radiotherapy only	6	1	4	—	1
Operation followed by radiotherapy	6	5	—	1	—
Radiotherapy followed by operation	7	6	—	—	1*
Total No.	19	12	4	1	2
%		63	21	7.5	
		84%			

*Did not undergo complete operation.

because of disease-containing nodes, and one did not undergo complete operation and died of nodal metastases.

Recurrence analysis. Recurrences were classified as local (affecting the vulva itself), regional nodal (inguinal-femoral nodes), or distant. We do not distinguish between superficial and deep inguinal-femoral nodes. Intrapelvic nodes are considered "distant" rather than regional.

In the palliative group (Table V), local control was obtained in 19 of 40 patients. Since palliative doses of radiotherapy are usually given, it is surprising that local control in this group was as good as it was; this underlines the radiosensitivity of vulvar cancers.

In the group with recurrence after operation, local control was obtained in only two. In the curative radiotherapy only group, three patients had local control with one having been treated by megavoltage irradiation and the other two by radium needle implant only. Two further radium needle implants resulted in local failure.

In the postoperative radiotherapy group, only one of six experienced failure of local control; this patient is alive with disease after further treatment with single-field radiotherapy. In the preoperative radiotherapy group, one patient experienced failure of local control, the recurrence developing in the vulvar region following operation. This recurrence was successfully treated by further radiotherapy to the involved area. All other patients completing preoperative radiotherapy and operation are alive without recurrence. It is emphasized, however, that two of these had diseased nodes and received further local radiotherapy. It is important to recognize that, for the six patients undergoing complete operation in group 5, histologic examination of the vulvectomy specimen revealed no residual disease, although one patient developed a local recurrence.

Analysis of complications. Acute complications were defined as those occurring during treatment and up to 3 months thereafter, although in many centers these would be regarded as radiation side effects. Chronic complications were those that developed after 3 months. In both cases, the arbitrary scale previously described was used to assess the severity.

For the acute complications depicted in Table VI, in one patient severe diarrhea persisted and became chronic and in one patient a severe skin reaction failed to heal.

Table VII documents chronic complications. Only four (6%) patients experienced persistence of "severe" posttreatment problems (leg edema, three; skin necrosis, one) and there were no cases of fistula formation.

Of much greater importance is a description of the complication rates in those groups where combined-

Initial stage (TNM grouping)						*Survival*		
I	*II*	*III*	*IVA*	*IVB*	*Unstaged*	*Alive at 5 yr*	*Deaths*	*Actuarial survival (%)*
0	4	8	13	3	12	5	31	17
1	1	2	1	—	4	4	4	56
3	—	3	—	—	—	2	3	50
1	1	4	—	—	—	3	1†	92
1	3	2	1	—	—			
6	9	19	15	3	16			
		54%						

modality treatment with curative intent was given (Table VIII). Regardless of whether radiotherapy was given before or after operation the rate of severe chronic complications was zero. In the postoperative treatment group, however, there were a total of eight acute complications in six patients, with four of these judged to be severe (skin, three; diarrhea, one). In contrast, in the preoperative treatment group there were six acute complications (five skin reactions), with only one judged to be severe.

Table IV. Node status

	Curative radiotherapy only (n = 6)			*Postoperative radiotherapy (n = 6)*		
Nodes	*T1*	*T2*	*T3*	*T1*	*T2*	*TX*
N0	2	—	—	—	1	—
N1	—	1	2	1	2	—
N2	—	—	1	—	1	—
NX	—	—	—	—	—	1

Comment

We believe the data presented support a reevaluation of radiation therapy for this disease. This is also suggested by other reports,[3-5] and indeed the negative opinion of radiotherapy entrenched in the literature[6] needs rethinking.

Carcinomas of the vulva are accessible and radiotherapy is often a curative modality at other sites of squamous malignancy. It is important, therefore, to examine those patients not only treated by more up-to-date methods of radiation therapy (megavoltage) but also treated with curative intent.

One premise that we have examined in this review is that radiotherapy and operation can be combined in a selective and hopefully optimal way. For patients with lesions of the posterior vulva (fourchette) or perineal lesions, the data suggest that local radiotherapy can effectively sterilize the tumor bed and that such a bed of tumor can be surgically removed in an attempt to prevent unnecessary radical and often permanent functional impairment. For patients in this group the added morbidity associated with an abdominal perineal resection is prevented with no apparent reduction of local control of disease. By restriction of the size of radiotherapy fields, high doses of radiation can be used without effecting a compromise of the normal tissues or the surgical results in terms of later planned radical vulvectomy. Should diseased nodes be encountered at lymphadenectomy, inguinal field radiotherapy can still be given although the exit dose from a preoperative perineal field may compromise deep pelvic node radiation therapy. It is certainly not established that cure is possible in patients with diseased deep pelvic nodes if additional radiotherapy is given, and we have abandoned routine deep node dissection.[1] The use of a single perineal portal has been described before in the literature,[7] and the use of preoperative radiation so that more extensive surgical procedures will not be required also has support.[8]

Postoperative radiotherapy resulted in effective control rates for a small group of patients. On the basis of diseased nodes and/or resection margins such patients would be expected to have a much lower survival rate than was obtained. Radiotherapy may well improve survival in such surgical cases; however, this series has shown that the incidence, duration, and severity of complications are appreciably raised (Table VIII). We have concluded, therefore, that for selected patients small or restricted volume preoperative radiotherapy is the optimal way to maximize the benefits of combined radiotherapy-operation.

The observations in and results of this study have led us to reexamine the optimal management of carcinoma of the vulva. The cure of vulvar cancers by radiotherapy alone, the "salvage" of patients with surgical failures in a fair proportion of cases, the ability to provide significant palliation, and the sterilization of primary tumor beds by preoperative radiotherapy (also commented on by Acosta et al.[2]) were provocative observations from this review.

Table V. Recurrence patterns

Treatment group	n	Recurrence			
		None	Local	Regional nodes	Distant
Palliative*	40	1	21*	9	9
Radiotherapy, surgical failure	9	2	4	2	1
"Curative" radiotherapy only	6	3	2	1	—
Total No.	55	6	27*	12	10
Combined modality groups					
Operation followed by radiotherapy	6	5	1	—	—
Radiotherapy followed by operation	7	5	1	—	1†
Total No.	13	10	2	0	1
%		77	15		8

*Includes persisting disease.
†Did not undergo complete operation.

Table VI. Acute complications

Treatment group	n	Infection		Skin reactions		Diarrhea	
		Mild-moderate	Severe	Mild-moderate	Severe	Mild-moderate	Severe
Palliative	40	2	—	4	8	1	1
Radiotherapy, surgical failure	9	—	—	3	—	1	1
"Curative" radiotherapy only	6	—	—	2	—	1	—
Operation followed by radiotherapy	6	1	—	1	3	2	1
Radiotherapy followed by operation	7	1	—	4	1	—	—
Total	68	4	—	14	12	5	3

Table VII. Chronic complications

Treatment group	n	Leg edema		Necrosis		Fibrosis		Diarrhea	
		Mild-moderate	Severe	Mild-moderate	Severe	Mild-moderate	Severe	Mild-moderate	Severe
Palliative	40	2	2	—	1	1	—	—	—
Radiotherapy, surgical failure	9	2	1	2	—	—	—	—	—
"Curative" radiotherapy only	6	—	—	—	—	—	—	1	—
Operation followed by radiotherapy	6	4	—	1	—	—	—	1	—
Radiotherapy followed by operation	7	2	—	—	—	1	—	—	—
Total	68	10	3	3	1	2	—	2	—

Traditional methods of operation involve disfigurement and possible loss or compromise of sexual function. Recent successes in the management of breast cancer by conservative surgical procedures and radiotherapy/chemotherapy exemplify the value of questioning traditional surgical methods in an attempt to gain less tissue morbidity for at least an equal chance of disease control and cure.

This study does not provide sufficient data for an unequivocal statement that a departure from standard radical vulvectomy and node dissection is warranted. It does, however, invite us to consider new treatment approaches. For example, would bilateral node sampling be a new departure point in management? Those patients with negative inguinal-femoral nodes might then receive radical dose external radiation therapy to the primary malignancy only. Local control of disease might be further enhanced by either the addition of

Table VIII. Complication rates—Combined treatment

	Complications			
	Mild-moderate		*Severe*	
Treatment	*No.*	*%*	*No.*	*%*
Operation followed by radiotherapy (<3 mo) (n = 6)				
Acute	4	67	4	67
Chronic	6	100	0	0
Radiotherapy followed by operation (n = 7)				
Acute	5	71	1	14
Chronic	3	43	0	0

conservative excision or the supplementation of radiotherapy with brachytherapy techniques. In this manner, particularly for the younger woman, clitoral sparing and better preservation of vulvar contours might be possible. For patients with clitoral involvement, local high-dose radiotherapy may provide a chance for preservation of function. Patients found to have diseased nodes at inguinal sampling might be selected to undergo radical surgical procedures with or without additional postoperative groin and/or deep pelvic node irradiation. The adjuvant use of systemic therapy such as cisplatin has yet to be considered as well.

We believe that, despite the mixed nature of the data, there is a need for an ongoing and thorough reevaluation of the role of modern radiotherapy techniques in vulvar cancer, more particularly in the role of such therapy combined with either traditional or more selective surgical procedures. That radiotherapy has no role in the curative management of vulvar cancer is a tenet that is no longer valid and requires an honest reappraisal in the settings of treatment policies and clinical trials.

Mr. John Spinelli, Division of Epidemiology, Cancer Control Agency of British Columbia, assisted with the statistical aspects of the study.

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Diabetes in pregnancy: A population-based study of incidence, referral for care, and perinatal mortality

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During 1979 and 1980 in Washington State, 260 infants (live births plus fetal deaths ≥20 weeks' gestation) were born to women with preexisting diabetes mellitus, the equivalent to a population-based incidence of 2.1 per 1000 total births. One quarter of these women had non-insulin-dependent diabetes prior to pregnancy. The perinatal mortality rate for all infants of diabetic mothers in this series was 108 per 1000, which was eight times the state perinatal mortality rate. Only 45% of births occurred in the five tertiary centers in the state, whereas 39% occurred in hospitals that had fewer than six deliveries per year complicated by overt diabetes. The mortality rate was slightly, but not significantly, lower among infants born in referral hospitals than among those born in primary-level hospitals. Congenital malformations accounted for 43% of the 28 perinatal deaths, and fetal losses between 20 and 27 weeks' gestation accounted for another 21%. During the 2-year study period there were only three cases in which antepartum care in nonspecialty centers may have contributed to a perinatal loss. (AM J OBSTET GYNECOL 1985;151:598-603.)

Key words: Diabetes mellitus, high risk, regionalization

It is well established that diabetes mellitus in pregnancy is associated with increased risk of fetal and neonatal mortality. For over 50 years numerous authorities have advocated that pregnant women with preexisting diabetes be managed by teams of specialized personnel, preferrably in centers where high-risk obstetric and neonatal services are available.[1] Recently, reports from several diabetes centers have documented that with early, rigorous, and coordinated perinatal management, perinatal mortality rates for pregnancies complicated by preexisting diabetes can be reduced to levels in the range of 35 to 46 per 1000 births.[2-4]

These results, however, may not be indicative of the experience of the general pregnant diabetic population, particularly of those women delivering outside of major medical centers. First, patients in diabetic centers may be atypical either because they are referred as a result of high-risk status or because they live in proximity to teaching hospitals. Second, the outcomes reported are undoubtedly due in large part to care by providers whose interest and expertise in the care of pregnant women with diabetes surpasses that of physicians who serve the majority of the general pregnant population.

To establish estimates of the incidence of pregnancy complicated by preexisting maternal diabetes and to measure perinatal risks for an unselected population of pregnant diabetic women, we undertook a population-based study of these pregnancies in Washington State. The study involved all but one obstetric service in the state and reflects the experience of over 95% of resident births during 1979 to 1980. The purposes of this paper are (1) to report an unbiased estimate of the population-based incidence of preexisting diabetes in pregnancy, (2) to describe the degree to which pregnant diabetic women were delivered in hospitals equipped to manage high-risk deliveries, (3) to analyze the perinatal mortality in an unselected population of diabetic pregnancies, and (4) to examine the degree to which suboptimal referral to specialty centers may have contributed to potentially preventable mortality.

Material and methods

The study population was selected from Washington State resident births (either live births or fetal deaths ≥20 weeks' gestation) with delivery in a hospital or birthing center during 1979 and 1980. Births that occurred out of state were excluded, as were births from one small hospital that declined participation in the study. Also, births in 1979 in two military hospitals were excluded because medical records were not available for that year. Altogether these exclusions equaled <5% of all births of Washington residents during 1979 and 1980.

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Table I. Pregnancies complicated by preexisting diabetes mellitus in Washington state, 1979-1980

Group	*No. of births*		*Incidence rate (per 1000 births)*		*Maternal age (mean ± SD)*	*Prepregnancy weight (mean ± SD)*
Noninsulin-dependent diabetes	69*		0.5		30 ± 7†	173 ± 43†
White Class B	45	191	0.4	1.6	28 ± 4†	143 ± 40
White Class C	71		0.6		24 ± 5	139 ± 26
White Class D	55		0.4		25 ± 5	139 ± 23
White Class R-F	20		0.2		26 ± 5	140 ± 22
Nondiabetic Control	816		—		25 ± 5	136 ± 29

*Includes two twin births.

†Different from controls ($p < 0.001$; unpaired t test).

To maximize the chances of finding and enumerating all births complicated by diabetes, several different methods were used to identify potential cases: (1) all birth and fetal death certificates for 1979 and 1980 were hand searched to look for any mention of diabetes; (2) each of the 88 obstetric services in the state that participated in the study was visited, and on-site inspection of record room indices and of labor, delivery, and nursery logs was performed to identify suspected cases; (3) obstetric and nursery personnel were asked to suggest additional potential cases.

To confirm the diagnosis in the suspected cases identified by these methods, the hospital charts of both women and newborn infants were reviewed. In five cases, medical charts could not be located, and these cases are not included in this report. For confirmed cases, detailed demographic, historic, and clinical data were abstracted from the hospital records. Comparable information was also collected on a sample of 812 nondiabetic control pregnancies that were randomly selected with the use of birth and fetal death certificate files as a sampling frame. All live births in the study were matched against State of Washington infant mortality records to detect any neonatal death (<28 days of age) which may have occurred subsequent to discharge from the hospital.

Statewide statistical data on resident births and fetal, neonatal, and infant mortality rates were obtained from the Washington State Center for Health Statistics.

All diabetic women who were treated with insulin prior to pregnancy were classified into the appropriate White Class on the basis of information in their hospital records. In the analysis that follows, patients in White Classes R, F, or R-F were grouped together. Women who were diagnosed as having diabetes prior to pregnancy but who were not treated with insulin prior to pregnancy were classified separately as having non-insulin-dependent diabetes mellitus. Pregnancies of women who demonstrated glucose intolerance only during pregnancy are not included in this report.

The hospitals in the state were classified according to a system devised by the Washington State Hospital Association and the Washington State Department of Social and Health Services. Level I hospitals included mostly small services, with capabilities for managing only uncomplicated deliveries and newborns. The 11 Level II hospitals in the state generally had over 1000 deliveries annually and were capable of handling most maternal and neonatal complications, including Rh isoimmunization, and infants of diabetic mothers and low–birth weight infants without serious respiratory or other complications. The five Level III hospitals were equipped to treat any high-risk pregnancy or newborn.

Results

Incidence. During 1979 to 1980, 260 births (including two twin births) in Washington were complicated by preexisting diabetes; this is equivalent to an incidence rate of 2.1 per 1000 total births (Table I). One quarter of these pregnancies (67) were complicated by non-insulin-dependent diabetes and 39% (26) of these women were treated with insulin during pregnancy. Women in White Class C represented the largest proportion of pregnancies among those treated with insulin prior to pregnancy (37%). Women with non-insulin-dependent diabetes were older and heavier than nondiabetic control women, and women in White Class B were also older than control women. There were no significant differences in the number of prior pregnancies when figures were adjusted for maternal age. The incidence of diabetes in pregnancy was uniform among county subdivisions in the state. No difference in incidence rates was found between urban and rural residents or between white and nonwhite women.

Level of hospital of delivery. The distribution of births by hospital level is shown in Table II. Among all state resident births, 23.5% occurred in tertiary centers. For each class of diabetic pregnancy, the proportion of births in tertiary hospitals was significantly higher than the state proportion ($p < 0.01$) and ranged from 36%

Table II. Hospital of delivery for births complicated by maternal diabetes compared with all hospital births* in Washington State, 1979-1980

		Hospital level					
		Primary (Level I)		*Intermediate (Level II)*		*Tertiary (Level III)*	
Group	*No. of births*	%	*n*	%	*n*	%	*n*
Non-insulin-dependent diabetes	69	33.3	23	30.4	21	36.2	25
White Class B	45	26.7	12	33.3	15	40.0	18
White Class C	71	12.7	9	47.9	34	39.4	28
White Class D	55	18.2	10	30.9	17	50.9	28
White Class R-F	20	5.0	1	10.0	2	85.0	17
Total diabetes	260	21.2	55	34.2	89	44.6	116
Total in state*	126,305	47.2		29.3		23.5	

*Excluding births as described in section on materials and methods.

Table III. Perinatal mortality associated with preexisting maternal diabetes in Washington State, 1979-1980

		Fetal deaths (≥20 weeks)			*Neonatal deaths (≤27 days)*			*Perinatal deaths (Fetal and neonatal deaths)*		
Group	*No. of births*	*n*	*Rate**	*Relative risk‡*	*n*	*Rate†*	*Relative risk‡*	*n*	*Rate**	*Relative risk‡*
Non-insulin-dependent diabetes	69	4	58	7.7	1	16	2.2	5	72	5.0
White Class B	45	2	44	5.9	2	47	6.6	4	89	6.1
White Class C	71	6	85	11.3	1	15	2.2	7	99	6.8
White Class D	55	4	73	9.7	4	78	11.1	8	145	10.0
White Class R-F	20	1	50	6.7	3	158	22.4	4	200	13.8
All diabetic births	260	17	65	8.7	11	45	6.4	28	108	7.4
All resident births	133,233	999	7.5	1.0	934	7.1	1.0	1933	14.5	1.0

*Per 1000 live births plus fetal deaths.
†Per 1000 live births.
‡Relative risk compared to all state births.

for pregnancies complicated by non-insulin-dependent diabetes to 85% among women in White Class R-F. On the other hand, 21% (55) of births compicated by overt diabetes were delivered in Level I hospitals, even though these hospitals are not well equipped to provide high-risk perinatal care.

Of the 38 hospitals in Washington in which pregnant diabetic women were delivered, 30 had less than six such deliveries per year during 1979 to 1980. Deliveries in these 30 hospitals, however, accounted for 39% of the diabetic births in this series. Seven hospitals had between 6 and 12 deliveries of pregnant diabetic women per year and accounted for 48% of study births. Only one hospital in the state averaged over 12 diabetic deliveries per year.

Mortality. No maternal deaths occurred among the study pregnancies. There were 17 fetal deaths (≥20 weeks' gestation), which was equivalent to a fetal death rate of 65 per 1000 births. This was more than eight times the rate for the state as a whole, or 7.5 per 1000 births ($p < 0.001$). There were 11 neonatal deaths in our series, resulting in a neonatal mortality rate (infant of <28 days of age) of 45 per 1000 live births, more than six times the overall state rate of 7.1 per 1000 live births, ($p < 0.001$). The perinatal mortality for all diabetics was 108 per 1000 births. Mortality was lowest for pregnancies complicated by non-insulin-dependent diabetes (72/1000 births) and highest among births of White Class R-F women (200 per 1000 births) (Table III).

When perinatal mortality rates were compared by hospital level, rates were highest in Level I hospitals (164 per 1000 births) and lowest in Level II and III hospitals (92 per 1000 births). These differences in perinatal mortality rates, however, were not statistically significant. Furthermore, when the expected number of deaths (adjusted for differences in White Class) were compared to the observed number of deaths for each level, there were still no statistically significant differences in mortality among the different hospital categories. Specifically, there were nine perinatal deaths in Level I hospitals compared to an expected frequency of 5.0, seven deaths in Level II hospitals compared to an expected 8.9, and 12 deaths in tertiary (Level III) hospitals compared to an expected 14.1. Of note, however, is the fact that fetal deaths constituted eight of nine perinatal deaths in Level I hospitals and seven of seven deaths in Level II hospitals, but only two of twelve deaths in the Level III hospitals.

Twelve perinatal deaths, 43%, were associated with lethal congenital malformations, primarily of the central nervous system and heart (Table IV).

An additional six deaths were fetal losses that occurred between 20 and 27 weeks' gestation. Two of these fetal deaths were associated with umbilical cord accidents (a true knot and a nuchal cord wrapped four times), one was due to abruptio placentae at 23 weeks, and one was associated with an episode of diabetic ketoacidosis at 25 weeks of gestation in a Class B woman. The other two early fetal deaths were unexplained. Both occurred at 25 weeks (one in a Class C and the other in a Class D woman), and both pregnancies had been uneventful until the time of the fetal deaths.

A description of the remaining 10 perinatal deaths is given in Table V. Of the three deaths in Level I hospitals, two were late fetal deaths in women who had undergone only limited antepartum fetal surveillance. The neonatal death occurred in a moderately premature infant who died at 5½ hours of age while waiting to be transported to the regional neonatal intensive care unit. This infant was suspected of having a hypoplastic kidney and congenital heart disease, factors which may also have contributed to the early death. Case 4 was an infant of a previously undiagnosed woman who was admitted with a stillborn infant and in ketoacidosis at 30 weeks' gestation. The remaining six cases underwent intensive antepartum care in Level II and III hospitals. Nevertheless, two fetal losses occurred at 29 and 37 weeks, and four neonatal deaths occurred in four severely premature infants delivered by emergency cesarean section despite the immediate availability of intensive neonatal care in the tertiary centers where they were born.

Table IV. Congenital malformations associated with perinatal mortality in diabetic pregnancies

Malformation	*No. of cases*	*Description*
Anencephaly	3	Non-insulin-dependent diabetes, 27 wk/ fetal death; White Class C, 28 wk, fetal death; White Class D, 22 wk, fetal death
Occipital encephalocele	1	White Class D, 33 wk fetal death
Cyclopia	1	White Class D, 36 wk neonatal death
Caudal regression, multiple	1	Non-insulin-dependent diabetes, 40 wk, fetal death
Heart defects	4	White Class B, 35 wk, neonatal death; White Class B, 36 wk, neonatal death; White Class D, 31 wk, neonatal death; White Class F, 40 wk neonatal death
Multiple malformations	2	White Class B, 33 wk/ fetal death; White Class D, 36 wk, neonatal death

Comment

In the 1976 Report of the National Commission on Diabetes estimated the incidence of overt diabetes in pregnancy to be between 1 to 5 per 1000 births. This estimate was qualified, however, by the admission that no acceptable population-based figures were available at that time.[5] Since then, a study based on birth registration records from Norway estimated a population incidence of 3 per 1000 births.[6] Another study, which was made in South Carolina and based on birth/fetal death certificates and hospital discharge abstracts, found an incidence rate of 5.9 per 1000 births.[7] Neither study, however, clearly differentiated between gestational diabetes and diabetes that predated pregnancy.

Unlike estimates based on deliveries in specific institutions, the incidence rates estimated in this study reflect an unselected, statewide population encompassing over 125,000 births during a 2-year period. The accuracy of these estimates, however, is dependent on the degree to which diabetes was correctly diagnosed and recorded in the records reviewed in this study. It seems unlikely that pregnancies complicated by preexisting insulin-dependent diabetes would not be recognized, and it is also unlikely that this diagnosis would not be recorded.

It is possible, however, that this study may have underestimated the incidence of pregnancies complicated by non-insulin-dependent diabetes. First, some women with non-insulin-dependent diabetes may have gone undiagnosed. Second, others may have been diagnosed during pregnancy and therefore have been misclassified as having gestational diabetes. Third, since any woman treated with insulin prior to pregnancy was classified in this study into White Classes B through R, women who were receiving insulin prior to pregnancy but were not truly insulin dependent (as defined by the

Table V. Late fetal deaths and neonatal deaths not associated with lethal congenital malformations

Case No.	*Hospital Level**	*Group*	*Gestational age (wk)*	*Cause of death and comment*
1	I	Non-insulin-dependent diabetes	38	Unexplained fetal death prior to labor; no antepartum surveillance
2	I	White Class C	35	Prematurity, RDS; infant died awaiting transfer; possible renal and cardiac defects.
3	I	White Class C	35	Unexplained fetal death prior to labor; hospitalized 3 times during pregnancy
4	II	White Class C	30	Fetal death following presenting episode of diabetic ketoacidosis in a newly diagnosed 18-year-old diabetic
5	II	White Class C	37	Unexplained fetal death; active antepartum surveillance
6	III	Non-insulin-dependent diabetes	33	RDS; emergency cesarean section for fetal distress; Apgar scores, 1 and 0
7	III	White Class D	30	RDS; emergency cesarean section for deteriorating preeclampsia
8	III	White Class R	29	Fetal death; preeclampsia
9	III	White Class F	31	RDS; cesarean section for deteriorating renal function
10	III	White Class F	28	RDS; emergency cesarean section for fetal distress; transferred to Level III care 3 days before delivery

RDS, Respiratory distress syndrome.
*I, primary; II, intermediate; III, tertiary.

National Diabetes Data Group[8]) were also not counted in the non-insulin-dependent diabetes category in this study. Nevertheless, a large number of these pregnancies, constituting 25% of the total, were identified. Although this class of diabetic pregnancies has received little attention in the literature, it is clearly a high-risk category: the perinatal mortality in this group was almost five times that in the general population ($p < 0.001$). These infants appear to be at increased risk for fatal congenital malformations: anencephaly and caudal regression syndrome were each associated with fetal deaths in this group, and tricuspid atresia occurred in another infant, resulting in a postneonatal death at 3 months of age. It is noteworthy that these malformations are similar to those for which infants of insulin-dependent-diabetic women are at increased risk.[9]

Although recent reports have documented that in some centers the perinatal mortality among diabetic pregnancies can be reduced to levels of <5%,[2-4] our results suggest that these achievements have not been matched in this unselected series of diabetic pregnancies, where perinatal mortality exceeded 10% and was 5 to 14 times higher than the overall state rate.

The perinatal mortality among diabetic pregnancies in Washington State was nearly identical to that found in the population-based South Carolina study[5]—108 versus 102 per 1000 births. However, closer examination of the two studies reveals substantial differences in the pattern of mortality. The neonatal death rate was lower in South Carolina than in Washington (8 versus 45 per 1000 live births), while the fetal death rate was higher. If only white South Carolina births are used for comparison (a reasonable subgroup since over 85% of Washington births are to white parents), the South Carolina perinatal mortality is reduced to one half of that found in Washington. The difference is due entirely to the neonatal mortality component. The reasons for the different patterns of perinatal mortality are not known but probably involve the fact that the South Carolina statistics included pregnancies complicated by gestational diabetes, which are known to have lower perinatal, and particularly neonatal, mortality than pregnancies complicated by preexisting diabetes. Very similar discrepancies are found when mortality rates from this study are compared to the rates reported from the Norway study[6] that, like the South Carolina study, included pregnancies complicated by gestational diabetes.

It is noteworthy that only 2 of the 12 (17%) perinatal deaths in tertiary hospitals were fetal deaths, whereas fetal deaths constitute 15 of the 16 perinatal deaths in Level I and II hospitals (94%). This difference in the proportion of fetal deaths may have been due to several factors: (1) some fetal deaths may have occurred prior to the time when women are customarily referred to specialists; (2) women with known fetal loss may have elected (or have been encouraged) to deliver in a local community hospital rather than in a specialty center; (3) antepartum surveillance may have been used more vigorously in tertiary hospitals to manage the timing of delivery, thereby preventing some fetal deaths but increasing the number of neonatal deaths. For example, it is possible that with more aggressive monitoring, the choice in a tertiary center would have been to deliver Cases 1 and 3 (Table V) electively prior to their deaths in utero. Conversely, had Cases 6, 7, 9, and 10 not been carefully monitored in tertiary settings, they may have died in utero rather than in a neonatal intensive care unit. These contrasts also illustrate the problems of extrapolating to populations the experiences of tertiary centers, where neonatal deaths tend to outnumber fetal deaths.[2-4]

During the study period only 45% of women with preexisting diabetes delivered in tertiary facilities. Standards have not been promulgated for the proportion of diabetic pregnancies that should be delivered in Level III hospitals, but in Norway, for example, 77% of diabetic births occurred in tertiary centers.[6] On the other hand, comparative data are not necessary to evaluate the finding that 21% of deliveries in this study took place in Level I hospitals. With the exception of emergency deliveries, virtually none should have occurred in these hospitals, since by definition they were not prepared to provide high-risk obstetric and neonatal care. The same might be true also of the 39% of births which occurred in hospitals with fewer than six diabetic births per year.

Almost half of the deaths in this study were due to congenital malformations that are known to develop in the first few weeks of fetal life.[9] Another 25% occurred before 30 weeks' gestation, that is, prior to the time when most women might be hospitalized and/or receiving intensive antepartum fetal surveillance. Of those deaths that occurred in the third trimester, only two or three *may* have been attributable to receiving care in facilities not equipped to manage diabetic pregnancies (Cases 1 to 3, Table V). It is conceivable that Cases 1 and 3 might have survived if more thorough antenatal surveillance of fetal well-being had been performed and had indicated the need for elective delivery before fetal death had occurred. Case 2 might have survived if neontal intensive care had been available immediately at birth. However, the probable co-existence of renal and cardiac malformations in this infant may have made long-term survival impossible under any circumstances.

It is important to stress, however, that these three perinatal deaths constituted, at most, only 11% of the perinatal mortality in this study. Far greater was the contribution of congenital malformations. Although it is not known whether etiology of these malformations is the same in infants of insulin-dependent and non-insulin dependent women, both groups of infants should be considered to be at high risk. Since these malformations can be detected early in pregnancy, intense prenatal surveillance for congenital malformations should be undertaken for pregnant women with insulin-dependent as well as non-insulin-dependent diabetes. Furthermore, recent reports have suggested that hyperglycemia in the early weeks of pregnancy may be associated with the development of congenital malformations and that careful periconception glycemic control may actually prevent their occurrence.[10, 11] If these findings are confirmed (one such study is presently underway[12]), our results indicate that in the future the greatest contribution to decreasing perinatal mortality among infants of diabetic women will depend, not on improved referral to tertiary centers or even on advances in technical obstetric care, but rather on motivating all diabetic women (both insulin-dependent and non-insulin dependent) who are contemplating pregnancy to seek medical care early, to maintain good glycemic control, and in effect to begin their "prenatal care" before conception.

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A comparison of Lamicel and prostaglandin E_2 vaginal gel for cervical ripening before induction of labor

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The efficiency and safety of Lamicel, a new synthetic cervical ripening agent, were compared with those of intravaginal prostaglandin E_2 gel in a group of 80 primigravid women about to undergo induction of labor. Lamicel caused less uterine activity and fetal distress than prostaglandin gel, although the induction-delivery intervals were similar in both groups. More normal deliveries occurred in the Lamicel group than in the prostaglandin group. It is suggested that Lamicel is a useful, efficient preinduction ripening agent that is safer than intravaginal prostaglandin gel. (AM J OBSTET GYNECOL 1985;151:604-7.)

Key words: labor induction, prostaglandin E_2, Lamicel

The significance of the initial condition of the cervix to the course of labor is well known. An unripe cervix at induction of labor leads to a long induction-delivery interval[1] and increases the number of forceps deliveries and cesarean sections.[2] Agents that have been used to ripen the cervix before induction of labor include laminaria tents,[3] oxytocin,[4] prostaglandins, both systemically[5] and locally,[6] and estrogen gels.[7] The most successful and widely used have been local prostaglandin preparations, applied either vaginally or into the extra-amniotic space. These agents are not without hazard, causing an increase in uterine activity and potentially stressing the fetus; intrapartum deaths have been reported as occurring during their use.[8]

Lamicel, a synthetic laminaria tent, is a polyvinyl alcohol polymer sponge, impregnated with 450 mg of magnesium sulfate and compressed to form a cylindrical tent 5 mm in diameter and 77 mm long. Strings are attached to one end to facilitate removal. When inserted into the cervical canal a Lamicel tent absorbs water and swells to four times its' original size, in the process changing from a rigid structure to a soft sponge. Lamicel has been shown to dilate the cervix in patients undergoing first- and second-trimester therapeutic abortions.[9-11]

In the study described below the efficiency and safety of Lamicel tents, when used to ripen the cervix before induction of labor, were compared with the properties of an intravaginal prostaglandin E_2 preparation.[12]

Methods

Eighty primigravid women were admitted to the trial. Criteria for entry were that the patient required delivery, a decision made by the consultant in charge of the case or his deputy, that the cervix was unripe (Bishop score <6), and that a decision had already been made by the obstetrician in charge of the case to use a ripening agent, regardless of the trial. A record was kept of the patient's age, gestation, and the reason for induction.

Table I. Indications for induction of labor

	Group	
Indication	*Prostaglandin (No. of patients)*	*Lamicel (No. of patients)*
Postmaturity	13	15
Maternal hypertension	16	16
Other maternal problems	2	0
Suspected fetal growth retardation	9	9

The patients were randomly allocated (with random number tables) to one of two groups, to receive Lamicel or prostaglandin gel. A vaginal examination was performed by one of three investigators and the state of the cervix assessed and scored.[13] The chosen ripening agent was then inserted, 4 mg of prostaglandin E_2 in a 4% cellulose gel into the upper vagina or a Lamicel tent into the cervical os. For the next 4 hours the uterine activity was continuously monitored, with the fetal heart rate, by external cardiotocography.

Four hours after insertion of the ripening agent the patient was again examined by the same investigator. The Lamicel tent was removed, the cervix was assessed and scored, and the forewaters ruptured. The presence or absence of meconium was noted. An intravenous infusion of Syntocinon was begun, unless the patient was already established in labor. Labor was managed in the normal way by the team on duty for that day. The individual investigators took no part in management decisions. After delivery a record was made

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Table II. Group characteristics

	Group			
	Prostaglandin		*Lamicel*	
	Range	*Mean ± SD*	*Range*	*Mean ± SD*
Maternal age (yr)	16-36	24.3 ± 5.2	17-35	24.1 ± 5.3
Gestational age (wk)	38-42	40.33 ± 1.33	38-43	40.40 ± 1.66
Infant's weight (kg)	2.42-4.32	3.33 ± 0.46	2.04-5.18	3.29 ± 0.56

of the induction-delivery interval, the occurrence of fetal distress or hypertonicity, the total dose of Syntocinon given, the type of analgesia used, the type of delivery, the fetal weight, and the Apgar score at 1 and 5 minutes.

Results and statistical analysis

The indications for induction of labor are shown in Table I; as can be seen, the two groups were effectively identical.

Table II shows the very close comparability between the groups in terms of maternal age, gestation, and infant's weight at birth.

Eighty-five percent of the women in the prostaglandin group had epidural analgesia as opposed to 78% in the Lamicel group. This difference was not statistically significant.

The incidence of increased uterine activity and fetal distress during the 4-hour ripening period is shown in Table III. In the group given prostaglandin, 31 patients had evidence of increased uterine activity; 22 developed regular, painful contractions, one a tonic contraction, and the remaining eight evidence of infrequent contractions on the tocograph without the patient being aware of pain (designated tightenings). Only eight of the patients in the group treated with Lamicel had increased uterine activity, all designated tightenings. The difference between the two groups was analyzed by the D test for comparing two proportions and a very highly significant difference demonstrated ($p < 0.001$).

None of the patients given Lamicel showed any evidence of fetal distress in the ripening phase. Three patients who had been given prostaglandin had fresh meconium in the liquor amnii at artificial rupture of the membranes and one had an episode of fetal bradycardia severe enough to necessitate immediate cesarean section, following a tonic contraction. This difference between the groups did not quite reach acceptable levels of statistical significance ($0.05 < p > 0.06$, Fisher's exact test).

In the group given prostaglandins the range of initial Bishop scores was 0 to 5 with a median of 2. After ripening, scores ranged from 0 to 7 with a median of 2. In the group given Lamicel the initial Bishop score range was 1 to 5 (median of 2) and the range after ripening was from 1 to 7 (median value of 2). Analysis of these results by the Mann-Whitney U test showed that there was no significant difference between the groups' value for initial Bishop score ($p > 0.05$, two-tailed test) but that the patients given Lamicel experienced significantly more ripening than those in the group given prostaglandins (one-tailed test, $p < 0.05$). During labor, between artificial rupture of the membranes and delivery, significantly more Syntocinon was used in the Lamicel group (t test, $p = 0.05$).

Table III. Uterine activity and evidence of fetal distress in the ripening phase

	Group		
	Prostaglandin (No. of patients)	*Lamicel (No. of patients)*	*Significance*
Uterine activity			
Contractions	22	0	
Tightenings	8	8	
Tonic contractions	1	0	
Total	31	8	$p < 0.001$
Fetal distress	4	0	$0.05 < p < 0.06$

Patients undergoing cesarean section were removed from the groups for the calculation of the induction-delivery intervals. There was no significant difference between these corrected groups; these data are shown in Table IV.

One tonic contraction occurred in a patient given prostaglandins. Fetal distress was designated minor or major on clinical grounds. Minor fetal distress indicated a fetal heart rate abnormality during labor sufficient to cause a fetal blood sample to be taken but not calling for any further action. Major fetal distress indicated a problem leading to intervention and delivery by whatever means appropriate. Analysis by Fisher's exact test showed a significantly greater number of patients with minor distress in the group given prostaglandins ($p < 0.05$) but no difference in major fetal distress. The total number of patients affected, however, was much greater in the prostaglandin group than in the Lamicel group ($p < 0.005$).

The numbers of patients having each type of delivery

Table IV. Measurements during labor

	Group		
	Prostaglandin	*Lamicel*	*Significance*
Syntocinon used (units ± SD)	7.15 ± 8.21	11.20 ± 9.93	p = 0.05
Artificial rupture of membranes–delivery interval (hr ± SD)	11.87 ± 4.93	10.71 ± 4.17	NS
Tonic contractions	1	0	
Fetal distress			
Minor	10	3	$p < 0.05$
Major	10 (4*)	5 (3*)	NS
Total	20	8	$p < 0.005$

*Cesarean sections.

Table V. Type of delivery

	Group		
Type of delivery	*Prostaglandin (No. of patients)*	*Lamicel (No. of patients)*	*Significance*
Normal	9	19	$p < 0.02$
Simple forceps	12	9	NS
Rotational forceps	9	6	NS
All forceps	21	15	NS
Cesarean section	10	6	NS

are shown in Table V. Significantly more normal deliveries occurred in the Lamicel group (D test comparing two percentages, $p < 0.02$). No significant differences existed between the groups when other types of delivery were considered, although similar trends were seen.

The Apgar scores of the infants at 1 minute after birth were in the range of 4 to 10 with a mode of 9 for both groups. Rank sum totals were used to calculate the standard normal deviate and no significant difference was found between the groups. At 5 minutes the range of Apgar scores in both groups was identical (39 with a score of 10 and one with a score of 8).

One patient in each group had a urinary tract infection in the puerperium and one patient in the Lamicel group had a pyrexia of unknown origin, a high vaginal swab showing no pathogens.

Comment

Prolonged labor is associated with an increase in both damage to the fetus and operative delivery rates. If the cervix is unripe at induction of labor, prolonged labor is common and in some cases the combination of the indication for induction and an unripe cervix may make elective cesarean section the treatment of choice. A simple, efficient method of ripening the cervix before induction is, therefore, clearly of use.

The most successful agents used to ripen the cervix have been prostaglandin- and estrogen-containing gels placed in the upper vagina or extra-amniotically. Although these agents ripen the cervix they may also cause an increase in uterine activity. As the patients are always in an "at risk" group this may cause stress to the fetus and intrapartum deaths have been reported during ripening with these agents.[8] Laminaria tents have been used and these are associated with less uterine activity than prostaglandin preparations, although an intrauterine death has been reported during use of laminaria.[14] The major problem with these tents is one of infection. In a recent trial using natural laminaria[15] 60% of the patients developed endometritis and 12% of the neonates died of septic complications.

Lamicel is a synthetic polyvinyl sponge containing magnesium sulfate, sterilized by cobalt irradiation. In this trial it has been compared with prostaglandin E_2 vaginal gel for preinduction ripening of the cervix. The evidence suggests that the agents are as effective as each other. The induction-delivery intervals were essentially the same in both groups. There was some evidence that the Lamicel improved the Bishop score more than the prostaglandin gel, but this measure is clearly open to bias. A double-blind design for this trial was not regarded as being ethical as it would have involved each patient in twice as many vaginal examinations as were strictly necessary. Consequently, the results of change in Bishop score must be regarded with caution.

Statistical comparison of the two groups did not demonstrate a difference between them in terms of cesarean section rate or in forceps delivery rate. There was, however, a trend in both of these categories toward less intervention in the Lamicel group and, when the normal delivery rates were compared, the Lamicel group had significantly more normal deliveries than the prostaglandin group. The increased intervention in the prostaglandin group could be due to lack of progress in labor or maternal or fetal distress. The similarity between the induction-delivery intervals in the two groups would suggest that lack of progress was not a major factor.

More than 50% of the patients receiving prostaglandin became established in labor before the end of the ripening phase and nearly 80% had at least some increase in uterine activity. None of the patients in the Lamicel group became established in labor in the ripening phase and only 20% had any measurable increase in uterine activity. This is almost certainly responsible for the difference between the groups in incidence of fetal distress during the ripening phase and may be responsible for the increase in fetal heart rate irregularities seen in the prostaglandin group during labor itself. The only two patients to have tonic contractions were both in the prostaglandin group.

It seems, therefore, that Lamicel is as effective as and safer than prostaglandin E_2 gel in ripening the cervix. No increase was found in the rates of sepsis when Lamicel was used. The one disadvantage was that it was occasionally more difficult to insert the tent into the cervix than to put prostaglandin gel into the vagina.

The mechanism of action of Lamicel is still uncertain but probably involves a direct action on the connective tissue of the cervix. Further work is being carried out to evaluate more accurately the extent of uterine activity caused by Lamicel and the role of the sponge and magnesium sulfate elements. This, together with histologic and electron microscopic studies of the treated cervix, may make the mechanism of action clearer.

Lamicel is manufactured by Cabot Medical, 2021 Cabot Boulevard West, Langhorne, Philadelphia, Pennsylvania, and was donated by James F. Morrin.

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Neonatal mortality in infants born weighing 501 to 1000 grams

The influence of changes in birth weight distribution and birth weight–specific mortality rates on neonatal survival

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We analyzed changes over time in neonatal mortality rates for infants born weighing 501 to 1000 gm. The decline in total mortality in this weight group due to improved small-group, birth weight–specific mortality was contrasted to the increase in mortality caused by changes in the birth weight distribution resulting from the care of smaller infants. Had the birth weight distribution remained unchanged, the total improvement in neonatal mortality for the entire 501 to 1000 gm group would have been substantially greater. (AM J OBSTET GYNECOL 1985;151:608-11.)

Key words: Low birth weight, neonatal mortality, birth weight–specific mortality

Neonatal mortality in infants born weighing between 500 and 1000 gm has received considerable recent attention.[1-5] These studies have focused on the changes in survival rates within this birth weight group and factors responsible for the observed changes. However, few of these reports have evaluated shifts in the birth weight distribution or investigated changes in mortality rates for smaller birth weight groupings within this birth weight range. Because the changes in birth weight distribution and changes in birth weight–specific mortality rates may be important in understanding changes in the total mortality rate for this birth weight group, the analysis described in this study was performed.

Material and methods

The study population comprised all infants with birth weights of 501 to 1000 gm admitted to the Regional Neonatal Intensive Care Unit at the University of Alabama in Birmingham from 1974 to 1983. The infants were grouped by year of birth into four birth weight categories: 501 to 700 gm, 701 to 800 gm, 801 to 900 gm, 901 to 1000 gm. The smallest infants were placed into a 200 gm interval because of the small number of cases. For this study a neonatal death was defined as one that occurred prior to discharge from the hospital. Birth weight–specific neonatal mortality rates were reported as the number of deaths per thousand liveborn infants cared for within that birth weight range.

Neonatal mortality rates were analyzed with nonparametric (Friedman's) and parametric analyses of variance. A significant ($p < 0.05$) decline in mortality across the years under investigation was found. Therefore we utilized a method developed by Kleinman et al.[6] and Williams and Chen[7] to apportion the changes in mortality to either (1) shifts in birth weight distribution or (2) improvement in birth weight–specific mortality. Two-year time periods were chosen for analysis because of the relatively small numbers of infants and large variation in mortality rates between years.

In the analyses of Kleinman et al.[6] and Williams and Chen[7] the total change in the neonatal mortality rate arises from the addition of two summed terms. The first term represents the reduction in neonatal mortality rates attributed to changes in birth weight distribution between two time periods (e.g., 1974-1975 versus 1978-1979). This term was calculated by summation of the difference between the proportion of infants born into each birth weight category multiplied by the earlier birth weight specific-neonatal mortality rates. The second term represents that portion of the decline in neonatal mortality rates attributed to improvements in the birth weight-specific neonatal mortality rate. This term was calculated from the summation of the difference in the birth weight–specific neonatal mortality rates of the two periods being compared multiplied by the proportion of infants born into the respective birth weight categories during the later period.

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Results

During the years 1974 to 1983, 697 infants with birth weights of 501 to 1000 gm were cared for. The change in the distribution of birth weights of these infants over time is shown in Fig. 1. The proportion of births in the 501 to 700 gm category nearly doubled over the 10-year period, whereas that in the 901 to 1000 gm birth weight group was reduced.

The neonatal mortality rate for each birth weight group for the years 1974 through 1983 is shown in Table I. The neonatal mortality rate for all infants born weighing 501 to 1000 gm fell rapidly from 1974 through 1979, but since that time the neonatal mortality rate has been relatively stable. When considered over the 10-year period, the change in neonatal mortality for the group as a whole was found to be statistically significant ($p < 0.05$). When each of the smaller–birth weight group categories were considered individually, a statistically significant decline in neonatal mortality over time was found for each group. However, the plateau noted for the group as a whole after 1979 was not evident in the smaller groups.

An analysis was performed to determine, over time, the portion of changes in the neonatal mortality rate which could be attributed to (1) shifts in birth weight distribution and (2) improvements in the birth weight–specific neonatal mortality rates. Because the neonatal mortality rate for infants born weighing between 501 and 1000 gm appeared to plateau after 1979, our data were analyzed for the time periods 1974-1975 through 1978 to 1979, and 1978 to 1979 through 1982-1983, as well as for the decade as a whole. Fig. 2 shows the neonatal mortality rates and the changes that occurred between these time periods.

In comparing the time periods from 1974-1975 to 1982-1983, the first section of Table II shows that there was a total improvement in neonatal mortality for infants weighing 501 to 1000 gm of 228 deaths per 1000 infants cared for. However, if the birth weight distribution had not changed, the improvement in mortality would have been 292 deaths per 1000. Therefore shifts toward smaller infants increased the mortality for the group as a whole by 64 deaths per 1000. Shifts in the birth weight distribution over the decade, with a greater proportion of the infants in the lower–birth weight groups, therefore caused a substantial reduction in the improvement in the neonatal mortality rate that could have been achieved had the birth weights remained stable.

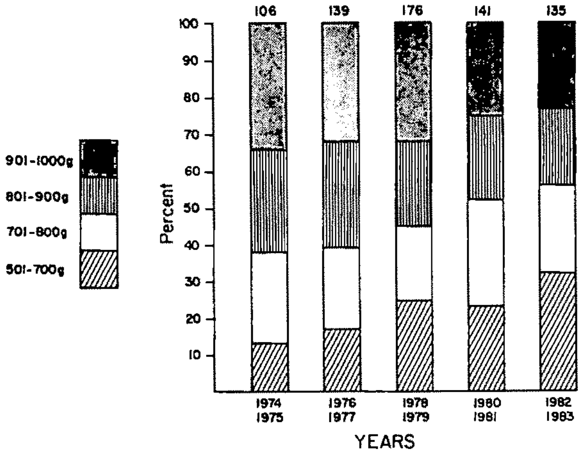

Fig. 1. The total number of infants born weighing 501 to 1000 gm under the care of the University of Alabama in Birmingham and the percent found in each birth weight group for 2-year periods from 1974 to 1983.

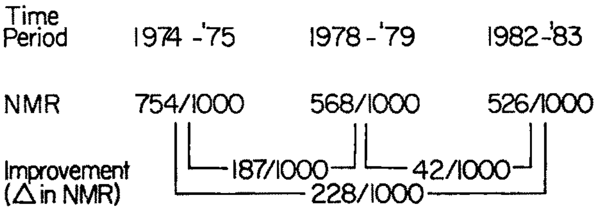

Fig. 2. The neonatal mortality rate *(NMR)* at the University of Alabama in Birmingham per 1000 infants born weighing 501 to 1000 gm who were cared for during 1974 to 1975, 1978 to 1979, and 1982 to 1983 and the improvement in mortality between the periods.

The middle section of Table II shows the analysis of the data for 1974-1975 through 1978-1979. Between these time periods there was a total improvement of 187 deaths per 1000 infants cared for. However, during this time, the changes in the birth weight–specific neonatal mortality would have resulted in a reduction of 214 deaths per 1000 infants cared for had the population not changed. Because of shifts in the population cared for to lower–birth weight newborns, the mortality rate was increased by 27 per 1000, yielding a total reduction in the mortality rate of only 187 per 1000. During this period there was an improvement in birth weight–specific neonatal mortality for each birth weight group.

The analysis of the data for 1978-1979 through 1982-1983 is presented at the bottom of Table II. During this time there was a decrease in neonatal mortality for the 501 to 1000 gm birth weight group of 42 deaths per 1000 infants cared for. The change in mortality was relatively small because the two factors being considered continued to move in opposite directions. From 1978-1979 to 1982-1983 there was an improvement in the birth weight–specific neonatal mortality rate of 83 deaths per 1000 infants cared for. For all the birth weight groups but one—801 to 900 gm—the trend in improved birth weight–specific neonatal mortality continued during this time. However, there was also a continued trend in our nursery toward lower–birth weight infants. This trend accounted for an increase in neo-

Table I. Neonatal mortality rates at the University of Alabama in Birmingham per 1000 infants cared for in various birth weight groups from 501 to 1000 gm in the years 1974 to 1983

Year	*501-700 gm*	*701-800 gm*	*801-900 gm*	*901-1000 gm*	*Total*
1974	1000	1000	750	700	800
1975	1000	778	857	375	698
1976	833	823	706	550	712
1977	1000	846	542	708	726
1978	850	650	500	500	616
1979	869	647	304	333	522
1980	895	666	553	347	605
1981	846	500	500	615	600
1982	769	555	533	294	566
1983	765	333	461	286	475

Table II. Source of decline in neonatal mortality from 1974-1975 to 1982-1983 by birth weight group. The improvement in the neonatal mortality rate (NMR) during the various time periods is attributed to either shifts in birth weight or shifts in birth weight–specific mortality rate

Causes of change	*501-700 gm*	*701-800 gm*	*801-900 gm*	*901-1000 gm*	*Total*
1974-75 vs. 1982-83					
Shifts in birth weight	−187	+1	+61	+61	−64
Shifts in birth weight–specific NMR	+74	+95	+62	+61	+292
TOTAL	−113	+96	+123	+122	+228
1974-75 vs. 1978-79					
Shifts in birth weight	−112	+30	+40	+15	−27
Shifts in birth weight–specific NMR	+34	+41	+96	+43	+214
TOTAL	−78	+71	+136	+58	+187
1978-79 vs. 1982-83					
Shifts in birth weight	−64	−22	+10	+35	−41
Shifts in birth weight–specific NMR	+30	+47	−23	+29	+83
TOTAL	−34	+25	−13	+64	+42

natal mortality of 41 deaths per 1000 infants cared for. The sum of these two numbers accounted for the decline in the neonatal mortality rate of only 42 deaths per 1000 infants cared for.

Comment

Neonatal mortality improved significantly for each of the birth weight groups between 501 and 1000 gm at this perinatal center during the last decade. At the same time there was a shift toward smaller and smaller infants. From 1974-1975 to 1978-1979 the improvement in birth weight–specific mortality dwarfed the negative influence of a shift toward lower birth weight. However, from 1978-1979 to the present, the shift toward smaller newborn infants masked a continuous, slow but steady improvement in the birth weight–specific neonatal mortality rate.

The reason for the shift toward lower–birth weight infants in this population was not studied. However, state vital statistics data for the same time period showed no major changes in the distribution of infants born weighing between 501 and 1000 gm. Since many of the very low–birth weight newborn infants who arrive at this center represent maternal/fetal or neonate transports,[8, 9] it is therefore likely that the observed shift in birth weight distribution was due to the increasing willingness of physicians throughout Alabama to refer high-risk, very low–birth weight fetuses and newborn infants for tertiary care. The smaller numbers of infants cared for in the 501 to 1000 gm range in recent years is explained by the recent opening of other perinatal centers throughout Alabama.

Authors of earlier studies have found it necessary to analyze neonatal mortality in specific birth weight groups in order to better understand the observed changes in neonatal mortality over time.[6, 7, 10, 11] Analysis of data by 500 gm birth weight categories for the entire population of newborn infants has proved useful. However, as shown in this study, analysis of smaller–birth weight groupings may be necessary to understand changes in neonatal mortality within a specific birth weight group over time. Similarly, accurate comparisons of neonatal survival between various institutions or geographic areas require precise knowledge of the birth weight composition of the group being studied.

Knowledge about differences in race, sex, the location of birth, gestational age distributions, and other population characteristics is also important to make appropriate comparisons between centers or to fully understand changes in mortality rates over time.[5, 8, 10] However, because of the recognized overwhelming importance of birth weight distribution to neonatal survival,[10, 11] changes in factors other than birth weight and neonatal mortality were not considered in this analysis.

In summary, beginning in 1978 to 1979, an apparent plateau in the neonatal mortality rate within the 501 to 1000 gm birth weight group was found to be the result of two factors moving in opposite directions. Continued improvement in birth weight–specific neonatal mortality rates has been documented. However, changes in the birth weight composition of the total group have moved the neonatal mortality rate in the opposite direction. Therefore, when describing changes in neonatal mortality rates over time for infants born weighing <1000 gm, changes in the birth weight distribution, if they occurred, should be documented.

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Cervical dysplasia: Association with sexual behavior, smoking, and oral contraceptive use?

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Information on possible risk factors of cervical dysplasia, including indices of sexual behavior, smoking habits, and the use of oral contraceptives was obtained from a case-control study in Ontario in which 250 cases of histologically proved cervical dysplasia and 500 age-matched neighborhood controls were interviewed. Initial analysis showed an increased risk associated with more than one sexual partner, an early age at first intercourse, current smoking, the use and duration of use of oral contraceptives, and education. However, the increased risk of use and duration of use of oral contraceptives and education was reduced after adjustment for indices of sexual behavior and smoking. This finding does not confirm previously reported large adverse effects of pill use. The relationship between smoking and cervical dysplasia might be explained by some agent in cigarette smoke which depresses the immune mechanism, thus permitting a sexually transmitted agent to result in abnormal cellular development that leads to the onset of cervical dysplasia. (AM J OBSTET GYNECOL 1985;151:612-6.)

Key words: Cervical dysplasia, sexual behavior, cigarette smoking, oral contraceptives, neoplasia

Because cervical dysplasia is considered to be a precursor lesion of cancer of the cervix, it should be expected that the risk factors for cervical dysplasia would be similar to those for cancer of the cervix. It is generally accepted that cervical cancer is a sexually transmitted disease and that women with cervical cancer tend to have first intercourse at an earlier age as well as multiple sexual partners.[1] These two factors explain most of the associations between cervical cancer and demographic characteristics such as high rates among divorced women and women in lower social classes.

Smoking and oral contraceptive use have also been implicated in studies of cervical cancer. Since Winkelstein[2] first suggested an association between smoking and squamous cell carcinoma, several studies[3-5] have supported this observation, some showing persistent elevation of risk after simultaneous adjustment for indices of sexual behavior or social status. Two studies of cervical dysplasia[6, 7] have also shown an association between smoking and cervical dysplasia. In 1983 Kelsey and Hildreth[1] reviewed the evidence of the association between the use of oral contraceptives and cervical neoplasia and concluded that the nature of the relationship remained uncertain. However, recent studies[8, 9] have suggested that long-term oral contraceptive use may increase the risk of cervical neoplasia. We report here the results of a case-control study that examines the relationship between dysplasia of the cervix and cigarette smoking and oral contraceptive use, taking into account other risk factors affecting the development of cervical dysplasia, specifically sexual behavior.

Material and methods

Cases eligible for this case-control study were selected from English-speaking women aged 20 to 59 years, residing in the Toronto area and attending the Dysplasia Clinic of the Toronto General Hospital between November 1, 1979, and March 31, 1981. For inclusion in the study it was necessary that the initial diagnosis of cervical dysplasia be histologically confirmed within 3 months of referral to the clinic. The diagnosis of dysplasia (mild, moderate, severe, or severe with carcinoma in situ) was determined by biopsy, dilatation and currettage, or hysterectomy, and all slides were reviewed by a single pathologist. No woman for whom the pathologist indicated that the lesion was carcinoma in situ only was eligible for the study. Of 284 eligible women, 250 were interviewed. Of the 34 women who were not interviewed, 12 (4%) could not be located because they had moved and left no forwarding address and 22 (7%) refused to participate in the study.

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Table I. Distribution of selected risk factors among 250 cases of cervical dysplasia and 500 controls

Factor	Cases		Controls		Estimates of relative risk
	n	%	*n*	%	
Years of education					
>13	89	36	227	46	1.0*
≤13	161	64	269	54	1.5†
Total	250		496		
No. of sexual partners					
1	40	16	203	44	1.0*
2-5	113	47	169	37	3.4‡
≥6	89	37	89	19	5.1‡
Total	242		461		
Age at first intercourse					
≥18 yr	125	50	350	73	1.0*
<18 yr	123	50	127	27	2.7‡
Total	248		477		
Smoking status					
Never smoked	53	21	202	40	1.0*
Ex-smoker	32	13	97	19	1.3
Current smoker	165	66	201	40	3.2‡
Total	250		500		
Current smokers (cigarettes/day)					
1-10	35	21	43	22	3.1‡§
11-20	49	30	69	34	2.7‡
>20	80	49	89	44	3.4‡
Total	164		201		
Oral contraceptive use					
Never	14	6	75	15	1.0*
Ever	236	94	425	85	3.0‡
Total	250		500		
Years of oral contraceptive use					
≤4	101	43	228	54	2.4†§
5-8	91	39	134	32	3.6‡
>8	42	18	58	14	3.9‡
Total	234		420		

Total figures vary because of missing values.
*Reference population.
†$p < 0.05$.
‡$p < 0.01$.
§Referent to never used.

Two English-speaking controls with intact uteri and no previous history of cancer were obtained for each case. Controls were matched to cases within 5 years of age and were also matched by neighborhood and type of dwelling (house or apartment) in an attempt to control the data for socioeconomic status. Potential controls were identified from municipal records that are updated every 2 years. Introductory letters were sent to 780 possible control participants. One hundred and sixty (20%) could not be located at the address given on the municipal records, 120 (15%) refused to participate, and 500 were then interviewed as potential controls.

Interviews with cases and controls were conducted at the woman's home or place of work, and controls were interviewed as soon as possible after the interview of the case. Each case and her associated controls were interviewed by the same interviewer who had no knowledge as to whether the women were cases or controls. A questionnaire sought information on social and economic status, gynecologic and obstetric history, details of sexual and smoking habits, and contraceptive practices.

Variables included in the analysis were indices of sexual behavior (number of sexual partners and age at first intercourse); smoking status (whether a woman currently smoked, was an ex-smoker, or had never smoked) and the number of cigarettes usually smoked per day; use of oral contraceptives (ever and duration); a measure of social status (years of education). Unmatched odds ratios were calculated for these variables by means of the method of Miettinin.[10] This led to more conservative (lower) estimates of relative risk than if matching had been preserved[11] but had the advantages of simplicity and fuller use of the data, especially when an item of information was not available for a case or

Table II. Estimates of relative risk for oral contraceptive use adjusted separately for life-style factors with use of matched logistic regression techniques (239 cases and 441 controls)

Model	*Estimates of relative risk*				
	Oral contraceptive use	*Oral contraceptive use + years of education*	*Oral contraceptive use + smoking status*	*Oral contraceptive use + age at first intercourse*	*Oral contraceptive use + No. of sexual partners*
Oral contraceptive use, ever vs. never	2.6*	2.5*	2.7*	2.3*	1.7†
Years of education, ≤13 vs. >13		1.6‡			
Smoking status					
Current vs. never			3.1*		
Ex-smoker vs. never			1.1		
Age at first intercourse, <18 vs. ≥18 yr				2.8*	
No. of sexual partners					
2-5 vs. 1					3.4*
≥6 vs. 1					6.9*

*$p < 0.01$.
†$p = 0.14$.
‡$p < 0.05$.

a matched control. The bulk of the analysis is therefore presented in this manner. Multivariate analysis with the use of matched logistic regression techniques was also performed on those women who had complete information on all variables.[11]

Results

Eighty-two percent of the 750 women interviewed were between the ages of 25 and 45, with the median being 29.0 for the cases and 30.0 for the controls. Cases and controls were neighbors, but a significantly lower proportion of the cases (36%) than of the controls (46%) had completed the equivalent of secondary school (Table I).

There were marked differences between the cases and controls with regard to indices of sexual behavior. Proportionately more cases than controls reported multiple partners and first sexual intercourse before the age of 18. When compared to women who had one sexual partner, women with two to five partners had a relative risk of 3.4 of developing cervical dysplasia, and the relative risk for those with six or more partners increased to 5.1. The relative risk for women who had first intercourse at the younger age was 2.7 when compared to those who had first intercourse when older.

A greater proportion of cases stated that they were current smokers, and they had a relative risk of 3.2 of developing cervical dysplasia compared to those who had never smoked. Among current smokers the cases smoked more heavily than the controls. More than 88% of all women participating in the study had used oral contraceptives at some time, but the proportion was greater among the cases who had also used oral contraceptives longer. These differences in the use of oral contraceptives yielded a relative risk of 3.0 of developing cervical dysplasia. There was some suggestion of an increased risk with increased duration of oral contraceptive use, with a relative risk of 2.4 for <4 years of use and a relative risk of 3.9 for >8 years of use. When age-adjusted relative risks were calculated for these risk factors, the estimates were essentially the same as those shown in Table I.

The duration of oral contraceptive use was associated with sexual history in both the cases and controls. With increasing duration of use, a higher proportion of women had multiple sexual partners, first intercourse at an early age, and currently smoked. There was no consistent pattern between the duration of use of oral contraceptives and education.

To investigate the role of oral contraceptives in more detail, matched logistic regressions were performed in which the effect of oral contraceptive use was adjusted separately for the confounding variables of smoking, sexual behavior, and education. Eleven cases and 59 controls were excluded from these analyses because of missing information for one or more of the variables of interest. It initially appeared that oral contraceptive users had more than twice the risk of developing cervical dysplasia (Table II). This risk was significant and stable when adjusted for education, smoking, and age at first intercourse separately, but when adjusted for the number of sexual partners per se, the odds ratio fell below 2.0 and was no longer significant.

When adjusted for all of these variables simultaneously, the significant relative risks were 2.9 for two to five sexual partners, 4.9 for six or more sexual partners, 1.9 for current smokers, and 1.7 for early age at first intercourse. The logistic regression analyses were repeated with the use of continuous variables, where appropriate, rather than the categorical variables shown, and the results were essentially the same.

In a more detailed analysis of these data that is not shown, four cases and 45 controls who had not had a cytologic screening test in the 3 years prior to interview were excluded in addition, and the results were unchanged.

Comment

The findings of an increased risk of cervical dysplasia associated with an increased number of sexual partners and an early age at first intercourse are consistent with those of studies of cervical cancer. We were fortunate to have encountered little resentment in requesting information regarding the number of sexual partners and age at first intercourse. Excluding 12 women (all controls) who stated that they had never had intercourse, only 36 (5%) of the women refused to answer these questions.

Our finding of an association between smoking and cervical dysplasia is similar to that of Harris et al.,[6] which was the only other study confined to women with cervical dysplasia. Like us, they did not report a dose-response relationship although this has been reported in studies of invasive cervical cancer.[3, 12, 13]

In our study, the increase in risk associated with oral contraceptives remained close to 2.0 after adjustment for age at first intercourse, cigarette smoking, or education but was no longer statistically significant when adjusted for the number of sexual partners. Because smoking, age at first intercourse, and the number of sexual partners appear to be acting independently, it does not seem possible to address a relationship between oral contraceptive use and cervical neoplasia without information on all these variables, particularly the number of sexual partners. Most studies reporting an association between cervical neoplasia and oral contraceptives have had no information on sexual behavior or had only indirect measures such as marital status or age at marriage.

Besides our own study, the only studies of which we are aware which look directly at sexual habits, oral contraceptive use, and cervical neoplasia are the case-control studies of Swan and Brown[14] and of Harris et al.[6] Swan and Brown reported no statistically significant association with oral contraceptive use after adjustment for sexual factors. Harris et al. found oral contraceptive use to be a significant risk factor after adjusting for sexual behavior, smoking, and other variables, with the greatest effect found among women who had used oral contraceptives for ≥10 years. Our study failed to find a similar effect in spite of the inclusion of women with long exposure (43 cases and 56 controls had used oral contraceptives for ≥3 years). Unlike the study of Harris et al., in this study the age at first intercourse remained an independent risk factor after adjustment for the number of sexual partners. The discrepancies between our findings and those of Harris et al. may possibly lie in the use of different sources of controls, since theirs were women receiving gynecologic care and ours were neighborhood controls. The discrepancies may also be due to different patterns of use of oral contraceptives in different countries at different time periods in women of different ages. Because the results in the study reported here were essentially unchanged when women who had not had a cytologic screening in the previous 3 years were excluded, we do not think that the lack of a significant association between cervical dysplasia and oral contraceptive use can be accounted for by a possible differing frequency of screening which may have occurred between users and nonusers of oral contraceptives.

In conclusion, the findings of this case-control study support the hypothesis that cervical dysplasia is the result of a sexually transmitted agent in view of the association seen with the multiplicity of sexual partners after controlling for other risk factors. The independent effect of age at first intercourse suggests that the cervix may be more vulnerable to a sexually transmitted agent at younger ages. Disappearance of the significant effects of the use and duration of use of oral contraceptives after adjustment for sexual factors and smoking does not support a possible large adverse effect of the pill,[8] but a study with a large sample would be required to rule out a more modest effect. In contrast, the persistence of the elevated risk for current smokers while other variables were simultaneously controlled indicates that the association with smoking may be real and not, as suggested by Skegg et al.,[15] due to a yet unrecognized association between smoking and sexual behavior. Various mechanisms have been suggested regarding the biologic effect of smoking on organs not directly exposed to cigarette smoke.[16, 17] Perhaps, as suggested by MacDonald,[18] smoking may depress the immune mechanism and allow a sexually transmitted agent to result in abnormal cellular development leading to the onset of cervical dysplasia.

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Hemodynamic alterations associated with amniotic fluid embolism: A reappraisal

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Experimental amniotic fluid embolism in animals produces profound pulmonary hypertension and acute cor pulmonale without evidence of left ventricular compromise. Authors reporting hemodynamic alterations associated with clinical amniotic fluid embolism have traditionally attempted to explain their findings within this experimental framework. A reanalysis of the five published cases of amniotic fluid embolism, which include hemodynamic data derived from pulmonary artery catheterization as well as a report of a sixth case suggests a hemodynamic interpretation different from the traditional one based on the animal model. Left ventricular failure is the only hemodynamic abnormality consistently observed in humans, and the published data are most readily explained on this basis alone. A theoretical model of hemodynamic changes accompanying amniotic fluid embolism that incorporates both experimental and clinical observations is presented. Therapeutic implications are discussed. (AM J OBSTET GYNECOL 1985;151:617-21.)

Key words: Amniotic fluid, embolism, hemodynamics

Entrance of amniotic fluid into the maternal circulation is associated with hypoxemia, circulatory collapse, and coagulation defects. Despite extensive investigation in animals and widespread clinical recognition in humans, the English literature contains only five published reports detailing central hemodynamic changes associated with amniotic fluid embolism in humans.[1-5] We present here a documented case of amniotic fluid embolism resulting in maternal death. Hemodynamic information was obtained within 70 minutes of the acute event. Our observations, as well as a reappraisal of data presented in previously published reports, appear to challenge traditional concepts of hemodynamic alterations associated with amniotic fluid embolism in humans.

Case report

A. B., a 21-year-old Mexican woman, gravida 2, para 0, abortions 1, presented to the Los Angeles County/University of Southern California Medical Center with a 39-week intrauterine pregnancy and spontaneous rupture of the membranes 6 hours prior to admission. Physical examination was within normal limits. The blood pressure was 98/60 mm Hg and pulse was 72 bpm. Vaginal examination revealed the presence of thick meconium and the cervix was 1 cm dilated. The patient was admitted to the fetal intensive care unit and oxytocin induction of labor was begun. Twelve hours later, the cervix was 4 cm dilated. She had received no medication within the previous 2 hours. During a period of minutes, the patient suddenly became agitated and tachypneic, then unresponsive, and suffered a respiratory arrest. The patient was promptly ventilated by mask and then intubated. An electrocardiogram revealed sinus tachycardia. During intubation with an oxygen flow through the Ambu bag of 8 L/min, the arterial blood gas values were: Po_2, 28 mm Hg; Pco_2, 52 mm Hg; base excess, −2; and pH, 7.27. The blood pressure was 90/35 mm Hg and pulse 136/bpm. The fetal heart rate tracing exhibited a prolonged bradycardia. As a result, the patient underwent emergency cesarean section with delivery of a 3360 gm infant with Apgar scores of 2 and 9. Umbilical arterial gas values were: Po_2, 10 mm Hg; Pco_2, 79 mm Hg; pH, 6.97; and base excess, −11. The infant subsequently did well. Duration of operation was 47 minutes and the estimated blood loss was 200 ml. During operation, with the patient intubated and with 100% F_{IO_2}, the arterial blood gas values were: Po_2, 103 mm Hg; Pco_2, 40 mm Hg; pH, 7.19; and base excess, −10. The diagnosis of amniotic fluid embolism was suspected and the patient was transferred postoperatively to the Women's Hospital intensive care unit. A flow-directed pulmonary artery catheter was placed 1 hour and 10 minutes after the respiratory arrest. Blood pressure was 90/70 mm Hg and pulse 160 bpm. Other initial hemodynamic parameters are detailed in Table I. A chest x-ray film was consistent with diffuse pulmonary edema. The patient was unresponsive to deep pain and the pupils were fixed and dilated. Caloric and doll's eye reflexes were negative and deep tendon reflexes were absent. Funduscopic examination revealed blurring of the optic disc margins. Hemodynamic and clinical findings were consistent with left ventricular failure and cerebral edema. Treatment was initiated with digoxin and man-

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Table I. Hemodynamic measurements in a patient with amniotic fluid embolism

Parameter	*Value*	*Normal range*
Mean pulmonary artery pressure (mm Hg)	29	10-17
Pulmonary artery diastolic pressure (mm Hg)	24	8-15
Pulmonary capillary wedge pressure (mm Hg)	19	5-12
Central venous pressure (mm Hg)	9	5-12
Cardiac output (L/min)	5.8	6-8
Systemic vascular resistance (dynes · sec · cm^{-5})	938	900-1200
Pulmonary vascular resistance (dynes · sec · cm^{-5})	138	150-250
Left ventricular stroke work index (gm m/m^2)	19	51-61

nitol. For the next 6 hours, the patient remained hemodynamically stable. A repeat arterial blood gas measurement showed a Po_2 of 352 mm Hg with 50% Fi_{O_2}. She then became mildly hypotensive and a dopamine infusion was begun with resolution of the hypotension. During the next 12 hours, the mean pulmonary artery pressure and pulmonary capillary wedge pressure fell and stabilized at 17 and 8 mm Hg, respectively. The central venous pressure and cardiac output remained unchanged. A computerized tomographic scan of the head showed marked cerebral edema and a small temporal lobe hemorrhage. Although hemodynamic status remained stable, consecutive electroencephalograms showed complete absence of cortical activity. The patient was removed from the respirator and died 36 hours after the acute event. At no time was there any evidence of coagulopathy. Blood drawn from the pulmonary artery contained fetal squamous cells, hair, and vernix. The patient's family refused autopsy.

Comment

Amniotic fluid embolism is a rare obstetric condition with an incidence between 1 in 20,000 and 1 in 80,000 pregnancies.[6, 7] Twenty-five percent to 50% of patients with amniotic fluid embolism die within 1 hour of the acute event and the overall mortality is reported to be up to 86%. The most common cause of early death is cardiovascular, traditionally attributed to pulmonary hypertension and acute cor pulmonale. Those patients not dying of hemodynamic compromise may experience, in 40% of cases, a consumptive coagulopathy and possibly fatal hemorrhage.[7]

Although resuscitative efforts were immediately instituted, initial blood gas assessment in our patient revealed profound hypoxia secondary to massive ventilation perfusion mismatch. Despite stabilization of hemodynamic status it appeared that death was due to acute cerebral anoxia.

Traditional explanations for the hemodynamic changes associated with amniotic fluid embolism have focused primarily on the development of acute pulmonary hypertension and cor pulmonale. This is thought to be due to a vasoconstrictor response of the pulmonary vasculature to the presence of amniotic fluid or particulate matter in amniotic fluid.[6, 7] The systemic hypotension often associated with this event has been attributed primarily to decreased left heart preload with diminished cardiac output.[6] However, in the pregnant ewe, Reis et al.[8] showed a direct effect of amniotic fluid on systemic vascular resistance which was unrelated to any change in cardiac output. In this animal model, infusion of amniotic fluid into the circulation produced, within seconds, a 90% increase in mean pulmonary artery pressure, a 60% rise in central venous pressure, and a 150% increase in pulmonary vascular resistance without a concomitant rise in pulmonary capillary wedge pressure or other evidence of left ventricular compromise. In all instances, pulmonary artery pressures had returned to baseline levels within 10 to 30 minutes after the injection. In these animals, pulmonary edema was never observed. A similar response and prompt resolution were also observed in dogs by Attwood and Browning.[9] Present concepts regarding hemodynamic changes with amniotic fluid embolism in humans are largely based on these experiments. However, a review of this and the five other published reports in humans that include similarly derived data reveals a different hemodynamic picture from that observed in these animal models.

In contrast to observations in experimental animals, all published cases of documented amniotic fluid embolism in human patients are similar and involve only mild to moderate increases in mean pulmonary artery pressure, a variable increase in central venous pressure, and a high pulmonary capillary wedge pressure with evidence of left ventricular failure (Table II). In our case, as well as the case of Moore et al.,[3] calculation of left ventricular stroke work index and the left ventricular stroke work index–pulmonary capillary wedge pressure ratio reveals marked depression of left ventricular performance according to the criteria of Sarnoff and Berglund.[10] In the latter case, an elevation of central venous pressure to levels in excess of the mean pulmonary artery pressure indicates either concomitant right ventricular failure or pulmonic stenosis. Calculation of left ventricular stroke work index is not possible from the data presented in the remaining reports. However, in the patient of Dolynuik et al.,[4] a

Table II. Hemodynamic measurements in our patient and others with amniotic fluid embolism

	Series					
Parameter	*Schaerf et al.*[1]	*Masson et al.*[2]	*Moore et al.*[3]	*Dolynuik et al.*[4]	*Duff et al.*[5]	*Clark et al. (this report)*
Mean pulmonary artery pressure (mm Hg)	20	31	27	23	–	29
Pulmonary artery diastolic pressure (mm Hg)		26			27	24
Central venous pressure (mm Hg)	9		33		15	9
Cardiac output (L/min)	5.8	3.5*	3.7†	2.6		5.8
Pulmonary capillary wedge pressure (mm Hg)	14	22	21	14	18	19
Pulmonary artery diastolic presure–pulmonary capillary wedge pressure (mm Hg)		4			9	5
Pulmonary vascular resistance (dynes · sec · cm^{-5})	83	206	86‡	277		138

*Determined by oxygen consumption method.

†Cardac index.

‡Calculated on basis of presumed body surface area of 1.5 m^2. An increase in body surface area would make this value even lower.

cardiac output of 2.6 in the face of a pulmonary capillary wedge pressure of 14 mm Hg strongly suggests the presence of significant left ventricular dysfunction. This observation was confirmed by a cardionuclear scan demonstrating depressed left and normal right ventricular ejection fractions. Likewise, both Schaerf et al.[1] and Duff et al.[5] reported left heart failure in their patients. Evaluation of clinical and hemodynamic data presented in each case is thus consistent with the presence of left ventricular failure. In light of the aforementioned animal studies, the mild to moderate increases in pulmonary artery pressures observed in all patients with amniotic fluid embolism have been interpreted as reflecting intrinsic pulmonary artery spasm secondary to the presence of amniotic fluid or debris. However, neither the ewes of Reis et al.[8] nor the dogs of Attwood and Downing[9] had any evidence of left ventricular compromise and, in the presence of left ventricular failure, this traditional interpretation of moderately elevated pulmonary artery pressures associated with amniotic fluid embolism is invalid.

Pulmonary artery diastolic pressure in man primarily reflects left heart end diastolic pressure. Elevation of pulmonary capillary wedge pressure causes a rise in pulmonary artery diastolic pressure of essentially the same magnitude. Thus, in patients with left ventricular dysfunction (including all published cases of amniotic fluid embolism), an elevation of pulmonary artery pressure may be solely a reflection of elevated left heart pressures.[11] On the other hand, in patients with intrinsic pulmonary vascular disease, the pulmonary artery diastolic pressure will exceed the pulmonary capillary wedge pressure. About 6 to 10 mm Hg is generally accepted as the minimal significant pulmonary artery diastolic pressure–pulmonary capillary wedge pressure gradient consistent with involvement of intrinsic pulmonary disease in the genesis of elevated pulmonary artery pressures.[11, 12] In patients with left ventricular failure, an increase in pulmonary vascular resistance to the range of 200 to 300 dynes · cm · sec^{-5} is also a common finding and may be viewed solely as a compensatory response to decreased cardiac output.[13]

The published data allow calculation of pulmonary vascular resistance and/or the pulmonary artery diastolic pressure–pulmonary capillary wedge pressure gradient in each patient (Table II). In each case, pulmonary vascular resistance is either normal or slightly elevated and well within the range consistent with left heart failure alone.[13] Only in the case of Duff et al.[5] could the pulmonary artery diastolic pressure–pulmonary capillary wedge pressure gradient be considered slightly elevated. A further clinical observation suggesting that the hemodynamic profile derived from animal data may not be entirely applicable to amniotic fluid embolism in humans is the absence of pulmonary edema in the animal model and its relative frequency (25% to 70%) in human patients.[7, 14] All of these considerations support our contention that left heart failure is of major pathophysiologic significance in amniotic fluid embolism in humans. Indeed, to date, it appears to be the only significant hemodynamic abnormality consistently documented in human patients.

Experimental and clinical hemodynamic observations in amniotic fluid embolism can best be reconciled by postulating a biphasic pattern of hemodynamic disturbance. In this model, the initial response of the pulmonary vasculature to the presence of amniotic fluid and other debris of fetal origin is intense vasospasm, producing severe pulmonary hypertension and profound hypoxia. The experimental work already cited, however, supported by our observation of rapid improvement in arterial blood gas values following the initial acute event, suggests that this change, while severe, would appear to be transient. This initial period of hypoxia and acute right heart failure may account for the 50% of patients who die of amniotic fluid embolism in the first hour. The transient nature of this initial response, completely resolved in the animal model within 30 minutes, possibly accounts for the inability to document significant pulmonary hypertension in humans. Our report represents the earliest hemodynamic data collected to date (70 minutes after the acute event); pulmonary arterial catheter placement occurred more than 10 hours after the acute event in most previously reported cases.

Those patients who survive this initial phase apparently experience a secondary phase of hemodynamic compromise primarily involving left heart failure and variable secondary elevation of pulmonary artery pressure with return of normal right heart function. This is the hemodynamic picture documented in humans in the literature. The degree of pulmonary edema often observed in conjunction with only moderate elevation of pulmonary capillary wedge pressure suggests a component of noncardiogenic pulmonary edema as well. This pattern is an example of the adult respiratory distress syndrome. This hypothesis does not exclude the possibility of some degree of residual pulmonary artery spasm. Such left ventricular compromise may be a consequence of the initial profound hypoxia or perhaps represents a direct effect of amniotic fluid on the myocardium. While the former mechanism seems more likely, very indirect support for the possibility of the latter is suggested by the observation in vitro of decreased myometrial contractility in the presence of amniotic fluid.[15] The possibility of a similar effect of amniotic fluid on myocardium is intriguing but unproved. Duff et al.[5] suggested that left ventricular dysfunction observed in one patient with amniotic fluid embolism may be due to pulmonary hypertension and acute right ventricular dilatation, resulting in displacement of the intraventricular septum and decreased left ventricular filling. However, as we have shown, left ventricular failure is consistently seen even in patients with only mild elevation of pulmonary artery and right heart pressures. Furthermore, as shown by Sasamoto et al.,[16] simple elevation of mean pulmonary artery pressure to levels of 24 mm Hg is well tolerated and produces no clinical, radiographic, or electrocardiographic evidence of cardiopulmonary compromise. Mean pulmonary artery pressures of this magnitude or slightly greater have been associated with profound hemodynamic compromise and death in patients with amniotic fluid embolism. Such an elevation of pulmonary artery pressure alone is of minimal hemodynamic consequence and appears to be secondary to, rather than the cause of, left heart failure.

While half of patients with amniotic fluid embolism die within the first hour, the literature suggests an additional 25% to 50% cardiovascular mortality in the ensuing 4 to 5 hours. Recommendations concerning therapy for such patients are varied and are primarily directed at therapeutic attempts to relieve pulmonary vasospasm.[6, 7, 14, 17] The evidence derived from human subjects, however, suggests that for those patients surviving long enough to receive therapy (other than cardiopulmonary resuscitation) pulmonary artery vasospasm and cor pulmonale are not major problems. Rather left ventricular failure and pulmonary edema, including a significant component of adult respiratory distress syndrome, may be primarily involved in hemodynamic instability and death in these additional patients. Thus prompt and adequate inotropic support may be the most important determinant of survival in this group of patients.

In summary, in the management of patients with amniotic fluid embolism, the following should be considered. First, during initial resuscitation, profound hypoxia due to massive ventilation/perfusion mismatch occurs. Therefore, every effort should be made to provide the highest possible level of oxygenation during resuscitation. In this manner, the subsequent consequences of cerebral and myocardial ischemia, as well as additional short-term acidosis–induced pulmonary artery vasospasm, may be avoided. Second, prompt institution of pulmonary artery catheterization for proper hemodynamic assessment and management appears warranted. The data available in humans suggest that simple assessment of central venous pressure is inadequate and may, in fact, be misleading. Third, adequate inotropic support, guided by hemodynamic parameters derived from the pulmonary artery catheter, must be provided. Should such invasive monitoring not be available, routine rapid digitalization would seem appropriate. In the presence of persistent hypoxemia, another, less arrythmia-producing inotropic agent such as dopamine or dobutamine may be indicated. Further, although not the subject of this report, awareness of and prompt attention to the development of consumptive coagulopathy is essential. Attention to these principles may help to improve survival of patients with amniotic fluid embolism.

Finally, given the very rare occurrence of amniotic fluid embolism and the high immediate mortality, series from any single institution detailing hemodynamic changes in such patients are unlikely. Additional reports, including all hemodynamic data necessary for complete calculation and analysis of indices of left and right heart function and pulmonary vascular resistance, are needed.

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Comparative study of ultrasonically guided percutaneous aspiration with local anesthesia and laparoscopic aspiration of follicles in an in vitro fertilization program

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Twenty patients from our in vitro fertilization program were randomly divided into two groups. Ten women underwent follicular aspiration during laparoscopy while they were under general anesthesia and 10 women had an ultrasonically guided follicular aspiration with local anesthesia. All patients had mechanical infertility, and ovulation was induced with human menopausal gonadotropins. In both groups the same aspiration system with a needle of 1.4 mm inner diameter and a continuous suction at 120 mm Hg were used. In the laparoscopy group the oocyte recovery rate was 82.5% and the fertilization rate 62.5%, with one twin pregnancy; in the ultrasound group the oocyte recovery rate was 75%, and the fertilization rate 61.9%, with a singleton pregnancy. Implications of these results are discussed. (AM J OBSTET GYNECOL 1985;151:621-5.)

Key words: In vitro fertilization, percutaneous follicle aspiration

The use for in vitro fertilization of human oocytes removed from patients shortly before ovulation has become a routine practice in many centers dealing with infertility. Considering the average low pregnancy rate of 20% reported worldwide[1, 2] it is obvious that most patients will have to submit to oocyte collection more than once before pregnancy is achieved. Therefore the success and acceptability of the in vitro fertilization procedure depend much upon its simplicity and safety.

The purpose of this study was to compare the conventional laparoscopic method for oocyte collection with general anesthesia to the recently described ultrasonically guided follicular aspiration with local anesthesia.[3, 4]

Material and methods

Patients. Twenty patients from our in vitro fertilization program were randomly divided into two groups. Ten women underwent laparoscopy while under general anesthesia and 10 had ultrasonically guided follicular aspiration while under local anesthesia. In the laparoscopy group (Table I) patients' ages ranged from 29 to 40 years (mean 34.3 years) and the duration of

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Table I. Results of oocyte collection by laparoscopy

Patient No.	*Age*	*Duration of infertility (yr)*	*Total of hMG ampuls*	*No. of follicles >15 mm*	*Dominant follicle diameter (mm)*	*Mean follicle diameter (mm)*	*Estradiol before hCG (pg/ml)*
1	40	10	12	3	20	18.0	880
2	34	9	19	3	20	18.6	1600
3	39	12	32	3	20	16.6	1200
4	32	5	12	3	19	17.3	1660
5	32	7	30	3	17	16.6	700
6	31	8	22	3	19	17.3	1000
7	29	6	18	3	21	18.3	650
8	35	10	12	4	20	18.0	1530
9	35	4	27	3	20	17.6	720
10	36	14	33	4	23	18.2	540
Mean	34.3	8.5	21.7	3.2	19.9	17.6	1050

Table II. Results of oocyte collection by the use of sonography

Patient No.	*Age (yr)*	*Duration of infertility (yr)*	*Total of hMG ampuls*	*No of follicles >15 mm*	*Dominant follicle diameter (mm)*	*Mean follicular diameter (mm)*	*Estradiol before hCG (pg/ml)*
1	32	5	26	3	18	16.0	1500
2	33	16	14	4	18	16.5	240
3	29	10	12	4	20	19.2	500
4	27	6	22	5	25	15.7	600
5	34	9	18	4	24	21.3	1650
6	40	17	21	1	19	16.0	300
7	27	6	12	1	20	20.0	520
8	38	19	26	1	18	18.0	520
9	35	9	27	3	20	18.3	1200
10	31	6	17	2	24	22.0	260
Mean	32.6	10.3	19.5	2.8	20.6	18.3	730

the infertility problem was 4 to 12 years (mean 8.5 years). In the ultrasound group (Table II) patients' ages ranged from 27 to 40 years (mean 32.6 years) and the duration of the infertility problem was 5 to 19 years (mean 10.3 years). In all cases the main cause of infertility was tubal occlusion or periadnexal adhesions and all had had previous tubal surgical procedures. Patients had spontaneous menstruation and a normal uterine cavity shown by recent hysterosalpingography; their husbands all had normal sperm counts and motility and had had a normal zona-free hamster egg penetration assay performed in our laboratory.[5]

Ovulation induction. Human menopausal gonadotropin (hMG) (Pergonal, Ikapharm, Israel) was used to induce controlled ovulation in both groups, starting with 2 ampuls a day from day 3 of the menstrual cycle and the dose was changed according to ovarian response.[6]

Monitoring follicular development. Daily 17β-estradiol assays were performed starting on day 5 of the cycle. Ultrasonic scanning of the ovaries started on day 1 to 3 of the cycle to rule out possible ovarian cysts before hMG was given. Scanning subsequently followed every estradiol assay by means of a dynamic sector scanner (Aloka, Mure, Nitaka-shi, Tokyo, Japan) with a 3.5 MHz transducer.

hMG therapy was stopped when the leading follicle reached a mean diameter of ≥17 mm, and 24 to 30 hours later, if the estradiol level was <2000 pg/ml, 5000 units of human chorionic gonadotropin (hCG) (Pregnyl, Organon, Holland) was administered intramuscularly.

Oocyte collection technique with use of ultrasound. For the percutaneous follicular aspiration we used the same Aloka sector scanner, a 3.5 MHz transducer, and the original biopsy attachment to guide the needle. The punctures were performed on days 11 to 15 of the menstrual cycle, 35 to 38 hours after the injection of hCG. Patients were admitted to the hospital on the morning of the aspiration. No premedication was used. The urinary bladder was rinsed with isotonic saline through a No. 14 Foley catheter and subsequently filled

No. of oocytes recovered	*No. of mature oocytes*	*No. of immature oocytes*	*No. of cleaved oocytes*	*Outcome*
2	2	—	2	—
2	2	—	2	—
—	—	—	—	—
2	1	1	1	—
3	2	1	1	—
3	2	1	—	—
3	3	—	3	Twin pregnancy
4	3	1	2	—
3	3	—	2	—
2	2	—	2	—
2.4	2.0 (83%)	0.4 (17%)	1.5 (62.5%)	

No. of oocytes recovered	*No. of mature oocytes*	*No. of immature oocytes*	*No. of cleaved oocytes*	*Outcome*
4	3	1	3	—
1	0	1	1	—
6	4	2	2	Pregnancy
3	2	1	2	—
1	0	1	1	—
1	1	0	1	—
1	1	0	0	—
1	1	0	1	—
3	2	1	2	—
0	0	0	0	—
2.1	1.5 (71.4%)	0.6 (28.6%)	1.3 (61.9%)	

with 300 to 500 ml of isotonic saline containing 5 ml of indigo carmine dye. A local anesthetic applied to the suprapubic area consisted of 10 to 15 ml of 0.5% bupivacaine hydrochloride, and an intravenous drip of pethidine, 1.5 mg/kg, and diazepam, 10 mg diluted in 100 ml of isotonic saline solution, was started.

Puncture of the abdominal wall was guided ultrasonically in the direction of the follicles and was made by a trocar and cannula of 1.6 mm inner diameter passed through the biopsy attachment. Once the distended bladder was entered, the trocar was removed and the aspirating needle inserted through the cannula. A stainless steel needle 23 cm long with an inner diameter of 1.4 mm and an outer diameter of 1.6 mm was used as recommended by Lopata et al.[7] for laparoscopic follicular aspiration. The needle had a female Luer-Lok fitting at the proximal end and shallow tracks were drilled along 6 cm of the distal end to improve ultrasonic visualization of the needle. The needle was connected to a sterile disposable mucus aspirator (Uno, Hundested, Denmark) with a 20 ml container, which was connected to a continuous wall suction at a pressure of 120 mm Hg and operated by a foot pedal. The entire aspiration system was rinsed with 37° C warm heparinized culture medium.

After insertion of the needle through the cannula into the bladder, a pause was made near the posterior wall of the bladder with readjustment of the direction so that the full length of the needle was seen in the same plane with the maximal follicular diameter. The distance between the needle tip and the center of the follicle was measured to avoid puncturing through the follicle, which could cause spillage of the follicular content. Subsequently the needle was sharply advanced to the center of the follicle and instant aspiration was begun.

The straw-colored fluid thus obtained was immediately examined under a stereomicroscope; if the oocyte was found, the needle was withdrawn into the bladder and suction was stopped until the next follicle was located and punctured. If the oocyte was not collected, the needle was left in place and several flushings with heparinized culture medium were done until the oocyte was found.

Oocyte collection technique at laparoscopy. The same interval of 35 to 38 hours between administration of hCG and follicular aspiration was used, no premedication was given, and a final scanning of the follicles by ultrasound was performed a few minutes before the laparoscopy. A three-puncture technique[8] was used with the patient under general anesthesia. The entire aspiration system, including the needle, was identical to the one used in the ultrasound group.

Culture media. The culture media were prepared and the fertilization technique described by Leung et al.[9] was used.

Embryo transfer. We applied the method described by Jones et al.,[10] using a 20-gauge Teflon tube inserted through a 12-gauge steel carrying tube, with the patient in the knee-chest position.

Results

In the laparoscopy group a mean of 21.7 ampuls of hMG per patient were used (range 12 to 33) (Table I) and in the ultrasound group a mean of 19.5 ampuls were used (range 12 to 27) (Table II). The mean estradiol levels at the time of hCG injection were 1050 pg/ml in the laparoscopy group and 730 pg/ml in the ultrasound group. The number of follicles >15 mm was 3.2 per cycle in the laparoscopy group versus 2.8 per cycle in the ultrasound group. The mean dominant follicle diameter and the mean follicular diameter at the time of aspiration were 19.9 and 17.5, respectively, in the laparoscopy group and 20.6 and 18.3 in the ultrasound group. There was a recovery of 24 oocytes from 29 follicles, giving a recovery rate of 82.7% by laparoscopy compared to 21 oocytes recovered from 28 follicles, which gives a recovery rate of 75% for the ultrasound procedure. Fifteen of the oocytes cleaved in the laparoscopy group, giving a cleavage rate of 62.5%, and one patient (No. 7) has a twin pregnancy. In the ultrasound group 13 oocytes cleaved, a cleavage rate of 61.9%, and one patient (No. 3) is pregnant.

In one patient of the laparoscopy group, there was no access to the ovaries (No. 3) because of extensive pelvic adhesions and in one patient of the ultrasound group (No. 10), two follicles were punctured but no oocytes were recovered.

No mechanical damage to oocytes was observed. No complications were encountered in either procedure, and the acceptability of the ultrasonically guided puncture with the use of local anesthesia was remarkably good.

Comment

The procedure of in vitro fertilization is being used for more and more indications starting with mechanical problems of women whose tubal function has been irreversibly damaged by ectopic pregnancies, infections, surgical procedures in the pelvis, or endometriosis. Included also are male problems such as oligospermia, male-female interaction causes of infertility (that is, hostile cervical mucus), antisperm antibodies, and, finally, unexplained infertility.

Ultrasonic monitoring of follicular growth is part of every in vitro fertilization program. The technique was described by Hackeloer et al.[11] who used a full bladder to facilitate examination of the size and number of ovarian follicles in patients receiving gonadotropin therapy.

The recent works of Lenz and Lauritsen[3] and Wikland et al.[4] who described the use of ultrasound to guide follicular aspiration for in vitro fertilization make it a simple outpatient procedure.

The risk of ultrasonically guided puncture seems to be low. Holm[12] reported only one complication, an accidental puncture of the gallbladder, in 2500 ultrasonically guided punctures of abdominal tumors. Lindgren (personal communication) using needles with an outer diameter ≤2 mm had no complications in 1400 punctures of abdominal tumors. In patients undergoing ultrasonically guided follicular aspiration for in vitro fertilization, Lenz and Lauritsen[3] reported only transient hematuria in 4 of 30 patients and Wikland et al.[4] reported transient hematuria for 1 day in two of 44 patients. We encountered no hematuria and no other complications among our patients.

The presence of pelvic adhesions, which increases the risk and can even prevent follicular aspiration by laparoscopy, is of aid in ultrasonically guided punctures because of the fixation of the ovaries.

The well-known hazards of laparoscopy with general anesthesia, such as cardiac arrhythmias and arrest, bowel lacerations, and severe hemorrhage, are avoided by the ultrasound procedure with local anesthesia. Because of its low risk and low cost, the ultrasound procedure facilitates repeated aspiration in multiple cycles. It can also be considered when only one follicle develops and the feasibility of laparoscopy is questionable. Our results show that there was no significant difference between the recovery rates in the two groups (82.7% by laparoscopy and 75% by ultrasound) and no significant difference in the fertilization rate (62.2% in the laparoscopy group and 61.9% in the ultrasound group), which did not differ much from fertilization rates presented in the recent literature—66.3% by Lopata,[13] 60% by Leung et al.,[9] and 68% by Laufer et al.[14] The 10% pregnancy rate in the present series was somewhat lower than our overall pregnancy rate of 13.5%.

Another conclusion that derives from our data is that it is possible that general anesthesia and abdominal inflation with carbon dioxide do not damage the oocytes nor does the hyperprolactinemia induced by anesthesia, since our fertilization rates were almost identical in both groups; on the other hand, the ultrasonically guided procedure does no more harm to the oocytes than does the laparoscopic aspiration.

In view of the reliability, efficacy, low risk, and low cost (only one third the cost of the aspiration procedure by laparoscopy in our institution) of ultrasonically guided percutaneous aspiration of follicles, we believe that it should be the first choice in oocyte collection for in vitro fertilization, whereas laparoscopy should be reserved for cases in which a diagnostic laparoscopy is required in combination with an attempt at in vitro fertilization.[1, 15]

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Nutritional status of patients with untreated cervical cancer

I. Biochemical and immunologic assessment

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A prospective nutritional evaluation of 78 patients with untreated cervical cancer was undertaken. Stage-related abnormal anthropometric measurements were present in 60% of patients. Abnormal biochemical nutritional measurements were found in 67% of patients. Thirty percent of these patients were anergic to usual skin test antigens. The overall incidence of protein-calorie malnutrition was 12.5%; however, the risk was stage-related. Measured parameters were usually adversely affected following a surgical procedure. Although surgical complications were few, the implications of these findings as to the appropriate method and timing of nutritional assessment as well as the possible benefit from nutritional support in malnourished patients with cervical cancer are discussed. (AM J OBSTET GYNECOL 1985;151:625-31.)

Key words: Cancer, cervix, nutrition

Recent assessment of anthropometric, immunologic, and biochemical nutritional parameters has allowed the detection and classification of patients with obvious or occult protein or protein-calorie malnutrition. While in this country individual physicians or hospitals rarely care for clinically starved patients, recent reports indicate that objective laboratory nutritional abnormalities suggestive of protein-calorie malnutrition may be present in as many as 50% of hospitalized patients.[1]

While any patient is at risk to develop a nutritional deficit,[1] those with cancer, regardless of age or tumor origin, may be at increased risk for nutritional deficiency secondary to anorexia, taste and smell abnormalities, inadequate oral intake, or early satiety secondary to central or peripheral stimulation by abnormal tumor-produced peptides.[2] These problems frequently occur in cancer patients who have an increased basal metabolic requirement or in whom tumor competes for important nutrients.[2,3] These nutritional problems are likely adversely influenced by altered oral intake associated with the depression and anxiety that commonly exist in cancer patients.

The association of malnutrition in patients with terminal cancer is not new; however, the implication of a possible dietary etiology in as many as 50% to 60% of cancers in women is recent.[4] Coexisting malnutrition adversely affects the prognosis of the cancer patient[5,6] and is associated with an increased risk of complications during therapy.[7,8] Unfortunately, nutritional deficiencies may be exacerbated by current therapeutic modalities.[9,10]

Despite the implications of these recent developments, there exist few reports that delineate the nutritional status of patients with gynecologic cancer.[11]

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Table I. Patient characteristics

Parameter	No.	%
Mean age (yr)	50.6 (range 24-81)	
Race		
Caucasian	46	58.9
Black	32	41.1
State of disease		
I	47	60.3
II	14	17.9
III	11	14.1
IV	6	7.7
Therapy		
Operation	40	51.3
Radiation	36	46.2
Chemotherapy	2	2.6

This lack of information is likely related to the fact that the usual gynecologic patient is young and "healthy" and, in general, there is little physician suspicion of the possibility of a nutritional deficit. Additionally, primary malignancy of the reproductive organs, with the exception of ovarian disease, rarely involves the gastrointestinal viscera and theoretically should have little impact on the individual's nutritional status. However, the sparse amount of nutritional literature concerning patients with gynecologic cancer may be in part related to the fact that current medical school curricula or postgraduate courses place little emphasis on nutrition and the majority of gynecologists have little or no exposure to the possible clinical impact of malnutrition.

This prospective evaluation was undertaken in an attempt to define the incidence of anthropometric and biochemical abnormalities in patients with cervical cancer hospitalized for initial evaluation and treatment.

Methods and material

Between August, 1981, and December, 1982, prospective anthropometric and biochemical assessments were performed on 78 patients with untreated cancer of the uterine cervix who were admitted to the Gynecologic Oncology service at the University of Alabama at Birmingham (Table I). A nutritional evaluation was performed within 24 hours of initial admission and was repeated on the fifth postoperative day and weekly during the remainder of hospitalization.

Anthropometric assessment included measurement of triceps skin fold and midarm circumference with calculation of the midarm muscle circumference by the formula midarm muscle circumference = midarm circumference − 3.14 triceps skin fold. Both measurements were obtained from the patient's less dominant arm. Weight for height in each patient was calculated and compared to standardized tables.

Biochemical assessment included the measurement of serum albumin (cellulose acetate electrophoresis), serum hemoglobin, total iron-binding capacity (bathophenanthroline procedure), serum zinc (atomic absorption spectrophotometry), and serum copper (atomic absorption spectrophotometry). When possible, urine was collected for the determination of creatinine height index.* Values were compared to normal levels previously established at this institution.

Immunologic assessment included measurement of total lymphocyte count and skin testing of delayed hypersensitivity with mumps (0.1 ml), *Candida* (0.1 ml of 1:1000 dilution), and *Trichophyton* (0.1 ml of 1:1000 dilution). The skin tests were interpreted at 48 hours as anergic (no induration), hypoergic (1 to 4 mm of induration) or normoergic (>5 mm of induration).

Results

Admission history indicated that 35% of patients had recently experienced weight loss. A history of this factor was frequently present in those patients with advanced disease, and patients with Stage III or Stage IV disease were more likely (χ^2: $p < 0.05$) to have had recent weight loss exceeding 10 pounds. No relationship between weight loss, age, or race was evident.

Anthropometric measurements obtained at the time of admission indicated mean values well above standard for weight/height ratio and triceps skin fold and below standard for midarm muscle circumference (Table II). Anthropometric assessments <80% of normal were found equally in triceps skin fold (10% of patients) and midarm muscle circumference (9% of patients). Substandard values were present in as many as 51% of patients, and only 40% of patients had all three assessments equal to or above normal. Forty percent of patients had a single abnormal parameter, 9% of patients had two abnormal values, and 12% of patients had three abnormal assessments. An analysis of stage of disease indicated that abnormal anthropometric measurements were significantly (χ^2: $p < 0.0125$) more common in those patients with advanced disease (Stages III and IV). However, there was no apparent relationship to age or race. Correlation of these three parameters suggested a direct correlation between weight/height ratio, triceps skin fold ($p < 0.001$), and midarm muscle circumference ($p < 0.001$).

Postoperative assessment of anthropometric parameters indicated that 45% to 58% of values decreased following operation (Table III). While the overall percentage of abnormal anthropometric measurements did not differ between admission and the fifth postoperative day, evaluation of changes in individual patients indi-

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*Reference list for the laboratory studies is available upon request.

Table II. Anthropometric measurement

	Standard	*Admission*	*% Below standard*
Weight/height (pounds)	100	127.9 ± 29 (57-218)	14
Triceps skin fold (mm)	16.5	22.8 ± 9 (2-51)	26
Midarm muscle circumference (cm)	23.2	23.1 ± 3 (16-33)	51

Numbers in parentheses are ranges.

Table III. Postoperative anthropometric measurement

			% Abnormal	
	Standard	*% Decreased*	*Admission*	*5 Days after operation*
Weight/height (pounds)	100	58	14	13
Triceps skin fold (mm)	16.5	48	26	29
Midarm muscle circumference (cm)	23.2	45	51	61

Table IV. Biochemical assessment

	Standard	*Admission*	*% Below standard*
Albumin (gm/dl)	3.0-4.5	3.8 ± 0.6 (1.5-4.8)	9
Total iron-binding capacity (µg/dl)	270-400	266.2 ± 63 (111-462)	50
Hemoglobin (gm/dl)	10-14	12.9 ± 1.7 (7-16)	5
Creatinine height index (%)	100	96.5 ± 32 (14-193)	60
Prothrombin time (%)	80-100	85 ± 17 (10-100)	24
Lymphocyte count (No./mm^3)	>1500	2449 ± 1254 (318-7847)	22

Numbers in parentheses are ranges.

cated a significant (Student's paired *t* test: $p < 0.005$) decrease in triceps skin fold following operation.

Analysis of the biochemical assessments indicated abnormal admission values in 5% to 60% of patients (Table IV). In this particular group of laboratory studies, only 33% of patients had all normal values, while 18% of patients had at least three abnormal parameters. Only serum iron ($\chi^2: p < 0.001$) and serum albumin ($\chi^2: p < 0.007$) demonstrated a stage-related decrease. With the exception of a lower hemoglobin value in black patients ($\chi^2: p < 0.016$), there was no apparent relationship between any of the other biochemical assessments with age or race. Although only 9% of patients had serum albumin levels <3.0 gm/dl, an additional 10% of patients had serum albumin levels <3.5 gm/dl. The presence of an abnormally low serum albumin level predicted the presence of an abnormally low total iron-binding capacity ($p < 0.001$).

Patients rarely exhibited low serum levels of copper or zinc (Table V). In fact, 45% of patients had an elevated level of serum copper. The mean copper/zinc ratio was 1.9 and patients with advanced disease were statistically ($\chi^2: p < 0.014$) more likely to have a higher copper/zinc ratio. Abnormally low zinc levels correlated with an abnormal weight/height ratio ($p < 0.004$), an abnormal midarm muscle circumference ($p < 0.03$), and an abnormal serum albumin level ($p < 0.005$).

Postoperative changes occurred in the majority of patients evaluated (Tables VI and VII). Evaluation of individual patients indicated a significant (Student's paired *t* test) postoperative decrease in serum albumin level ($p < 0.001$), total iron-binding capacity ($p < 0.001$), total lymphocyte count ($p < 0.05$), and serum iron level ($p < 0.05$).

Results of delayed hypersensitivity testing indicated that 30.2% of patients were anergic or hypoergic to all

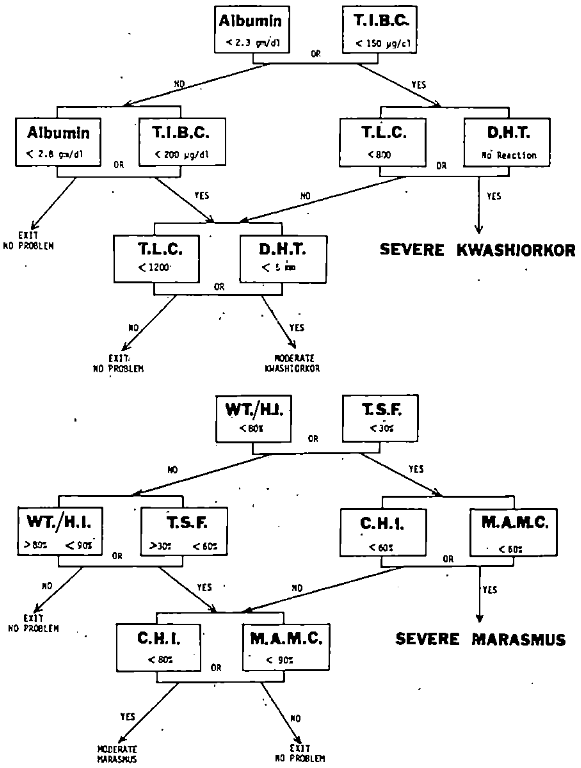

Fig. 1. Algorithm used to determine the presence of marasmus by means of weight/height ratio *(WT./H.I.)*, triceps skin fold *(T.S.F.)*, creatinine height index *(C.H.I.)*, or midarm muscle circumference *(M.A.M.C.)*. The presence of kwashiorkor was determined by use of serum albumin, total iron-binding capacity *(T.I.B.C.)*, total lymphocyte count *(T.L.C.)*, and results of delayed hypersensitivity testing *(D.H.T.)*.

antigens studied. In this group of patients there was no apparent relationship between failure to respond to skin testing and stage, age, or race; however, there was an apparent correlation with an abnormal serum albumin level ($p < 0.007$).

According to these values, patients were classified (Fig. 1) as to the presence or absence of protein-calorie malnutrition. No patient had severe protein-calorie malnutrition; however, 10 patients (12.5%) in this study had evidence of moderate protein-calorie malnutrition. The incidence of marasmus and kwashiorkor was equal. The incidence of protein-calorie malnutrition increased significantly ($\chi^2: p < 0.004$) in patients with advanced stages of disease. Four percent of patients with Stage I, 20% of patients with Stage II, 17% of patients with Stage III, and 60% of evaluable patients with Stage IV cervical cancer had evidence of protein-calorie malnutrition. The presence of protein-calorie malnutrition correlated with a history of recent weight loss ($p < 0.0002$) but not with age or race. The nature and selection of candidates for operation at this institution precluded the evaluation of a possible relationship between protein-calorie malnutrition and postoperative morbidity.

Table V. Assessment of minerals

	Standard	*Admission*	*% Below standard*
Zinc (μg/dl)	60-160	84 ± 16 (45-120)	8
Copper (μg/dl)	80-160	160 ± 40 (88-285)	0*
Iron (μg/dl)	42-135	55 ± 35 (7-240)	39

Numbers in parentheses are ranges.
*45% above standard.

Comment

The unstated goal of the gynecologic oncologist is to offer curative therapy and to minimize treatment morbidity. The usual armamentarium consists of surgical procedures, radiation therapy, or the administration of cytotoxic drugs used individually or in combination in an effort to effect a cure. These therapies often stress the physiologic reserves of the patient and therapeutic success will depend on the physician's ability to support patients with appropriate antibiotics, blood component therapy, and other intensive care or monitoring techniques. While these ancillary therapies are commonly used, only recently has the importance of nutritional evaluation and nutritional support been recognized. The implications of cancer-related malnutrition or cachexia, the sole cause of death in as many as 23% of cancer patients,[12] is apparent. However, only recently have physicians realized the adverse impact of most types of cancer therapy on the patient's nutritional status.[9, 10] The presence of malnutrition, regardless of the etiology, can have dramatic adverse effects on the musculoskeletal, cardiovascular, hepatic, renal, pulmonary, or central nervous system[5] and any nutritionally related problem may result in an altered therapeutic decision that may lessen the chance for cure or increase the risk of treatment-related complications.

To date, a standardized nutritional evaluation has been slow to evolve and no single abnormal laboratory parameter can be considered synonymous with malnutrition. More important, the evaluation of a number of anthropometric, immunologic, or biochemical determinations may be necessary in order to detect or predict the risk of complications in the malnourished.[13] In fact, the incidence of abnormal values depends on the intensity of investigation.[14] In the present study, the majority (60%) of patients had at least one abnormal biochemical or anthropometric value; however, the incidence of protein-calorie malnutrition with the use of multiple parameters was only 12.5%. The fact that many patients have many individual laboratory or anthropometric abnormalities is in agreement with previous reports[14, 15] and suggests the importance of analyzing multiple parameters. While current treatment

Table VI. Postoperative biochemical assessment

	Standard	% Decreased	% Abnormal: Admission	% Abnormal: 5 Days after operation
Albumin (gm/dl)	3.0-4.5	85	9	55
Total iron-binding capacity (μg/dl)	270-400	89	50	80
Hemoglobin (gm/dl)	10-14	60	5	23
Creatinine height index (%)	100	60	60	36
Prothrombin time (%)	80-100	70	24	50
Lymphocyte count (No./mm^3)	>1500	70	22	36

Table VII. Postoperative assessment of minerals

	Standard	% Decreased	% Abnormal: Admission	% Abnormal: 5 Days after operation
Zinc (μg/dl)	60-160	5	8	5
Copper (μg/dl)	80-160	0	0	0*
Iron (μg/dl)	42-135	89	39	85

*30% above standard.

does not allow us to relate nutritional status to morbidity or prognosis, this report suggests a frequent occurrence of nutritional abnormalities and implies the need for continued monitoring and correlation of nutritional status to allow the delineation of those nutritional studies that are important in the prediction of individual patient outcome, following treatment for cervical cancer.

Visceral protein synthesis, turnover, or loss associated with hepatic disease, metastases, or renal or other coexisting diseases is complex and the elimination of patients with these diseases may have contributed to the apparent lower overall incidence of malnutrition in patients with gynecologic cancer when compared to that in reports on patients with tumors of other origins.[6, 15] While these exclusions may not seem logical, the effect of significant liver disease on visceral protein synthesis and renal disease on creatinine excretion (for calculating creatinine height index) are but two examples illustrating the problems of nutritional assessment of patients with major or superimposed multiple organ system disease. The elimination of these patients probably decreased the apparent incidence of malnutrition; however, it was our intent to evaluate prospectively the nutritional status of those patients having only cancer. This exclusion is not intended to deny the importance of multiple organ disease as patients with cervical cancer not infrequently have other coexisting medical disease that subjects them to the development of malnutrition. However, pretreatment abnormalities are apparently frequent in patients without other disease, and in the presence of other abnormalities it becomes imperative that the gynecologist evaluate these possibilities when interpreting nutritional assessment parameters.

The reported findings of a significant incidence of stage-related protein-calorie malnutrition is in disagreement with at least one recent report.[11] The inclusion of anthropometric parameters in our report may partially explain this difference. Although this inclusion may be controversial, we feel the evaluation of anthropometric measurements is justified in that abnormal triceps skin fold, a measure of body fat, and abnormal midarm muscle circumference, a measure of body protein, indicate the presence of protein-calorie malnutrition, are less affected by concurrent disease, and are reported to be predictive of nutritionally related adverse effects of therapy.[1, 14] The stage-related incidence of protein-calorie malnutrition may be surprising in a malignancy that rarely involves the gastrointestinal tract; however, advancing stage is associated with an increased tumor burden which may result in an adverse nutritional effect in a manner other than altered nutrient absorption. In fact, malabsorption may not play an important role in cancer malnutrition, even in patients with gastrointestinal tumors.[5, 15]

The selective or physician bias in the nature of cervical cancer treatment, involving radiation or operation, might suggest that the presence of malnutrition is less predictive of surgical complication because those patients with advanced disease, medical illness, or malnutrition are not usually deemed candidates for operation and often receive radiation. To suppose that patients receiving radiation should undergo a less extensive nutritional evaluation because they are not faced with a physiologic stress is incorrect. Recent information suggests that radiation therapy is associated with weight loss and marked nutritional stress.[9] This report suggesting a high incidence of protein-calorie malnutrition in patients destined to receive radiation therapy justifies the importance of nutritional screening, as support of those deficient or marginally normal patients may not only decrease the risk of complications but also decrease the chance of treatment interruption and allow for the completion of a curative treatment regimen.[16, 17]

The finding that weight loss was significantly correlated with other factors associated with protein-calorie malnutrition reiterates the importance of an accurate, pretreatment historical evaluation. Treatment morbidity is increased in patients with weight loss[5] and the presence of this historical fact should elicit the need for further nutritional evaluation in order that appropriate support may be instituted.

Obesity should not be considered synonymous with adequate nutrition; in fact, its presence may mask or obscure the presence of malnutrition. On the other hand, abnormally low weight/height ratios should alert the physician as to the necessity and possible benefit of further nutritional evaluation. The results of this study suggest that, at initial diagnosis, the mean weight/height ratio of patients with cervical cancer is well above normal. Importantly, abnormalities of this easily obtained inexpensive measurement correlate with abnormal anthropometric measurements suggesting decreased fat (triceps skin fold) or protein (midarm muscle circumference) stores. Perhaps this assessment should be performed routinely at initial treatment as a part of nutritional screening. On the other hand, obese patients, even with early stage disease, are less likely to receive radical surgical treatment and, if nutritional assessment is not performed at initial diagnosis, many patients may be tilted into a malnourished state and suffer more adverse consequences of radiation or perhaps fail to complete therapy.

Although older patients may be predisposed to the development of malnutrition,[5] in this subgroup of patients with cervical cancer, age was not apparently related to abnormal nutritional studies in either individual assessments or the diagnosis of protein-calorie malnutrition. This information becomes important to the clinician in decisions to implement nutritional support prior to surgical therapy. Patients of all ages are subject to deficits and require appropriate nutritional evaluation, as pretreatment nutritional support will benefit only those patients with significant deficits.

Altered immunocompetence is commonly present in patients with cancer,[18] although it is not clear if anergy is a cause or an effect of the tumor burden. Regardless, abnormal immunologic studies, particularly an altered response to delayed hypersensitivity testing, are associated with protein-calorie malnutrition, appear to be prognostically important, and are associated with increased treatment morbidity.[7, 15, 18] In this report, 30% of patients failed to demonstrate an adequate response to any of the tested antigens. Thus a significant proportion of patients are at risk to develop infectious or other morbidity. The fact that this deficiency was not related to stage, age, or protein-calorie malnutrition is contrary to one report[15]; however, this lack of correlation may be related to the exclusion of patients with concurrent illness or infection, both of which are known to adversely affect delayed hypersensitivity testing.[19] Other factors such as abnormal serum iron or zinc levels reported to be associated with abnormal delayed hypersensitivity testing,[20] apparently were not correlated with cervical cancer in these patients. The presence of anergy in a significant percentage of patients without concurrent disease suggests the need for pretreatment evaluation in an attempt to identify possible risks and to allow proper supplementation regardless of the modality of therapy to be instituted.

A large proportion of these patients have an elevated serum copper level. While this is the initial report of elevated serum copper levels in patients with cervical cancer, it is in agreement with previous reports of patients with other cancers.[21] The stage-related abnormalities in zinc/copper ratios confirm one recent report[22]; however, the significance of elevated copper levels or stage-related abnormalities remains undetermined. Future investigation of the role of these minerals, both as a marker for malignant disease and as an indication of response to therapy, is warranted.

Lowered postoperative laboratory values were almost universal in those patients receiving surgical therapy. Although significant individual variations were prominent, these changes make it imperative that future studies of nutritional status delineate the time of nutritional evaluation. The synthesis or turnover of specific proteins or vitamins is likely affected by surgical stress and perioperative manipulations. Measureable parameters may be significantly altered by volume replacement or transfusion therapy. This finding validates the need for early initial assessment as even short-term hospitalization may have an adverse affect on measureable nutritional parameters.[23]

In conclusion, a large proportion of patients with cervical cancer have abnormal anthropometric, immunologic, or biochemical parameters prior to treatment. An additional number of patients have a marginal but "normal" nutritional status and may be pushed into a malnourished state during treatment. Stage-related abnormalities in triceps skin fold, midarm muscle circumference, serum albumin levels, and serum iron levels are apparent and, with the use of multiple parameters, the overall incidence of protein-calorie malnutrition is 12%; however, the incidence increases dramatically in a stage-related manner. Anergy was present in 30.2% of patients, suggesting that a significant proportion of these patients are at risk for infectious morbidity. Postoperative alteration in many of these parameters suggests the need for documentation of the timing of nutritional evaluation in subsequent reports.

Future investigation should focus on treatment-related morbidity as well as the prognostic impact of malnutrition in patients with cervical cancer.

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Nutritional status of patients with untreated cervical cancer

II. Vitamin assessment

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Prospective vitamin assessment was undertaken in 78 patients with untreated cancer of the uterine cervix. At least one abnormal vitamin level was present in 67% of patients while individual levels were abnormal in as many as 38% of patients. Significantly lowered levels of plasma folate, vitamin A, and vitamin C were present. Although most of these vitamins did not correlate with other parameters of protein-calorie malnutrition, the possible preventative and treatment effects of these vitamins require additional investigation. (AM J OBSTET GYNECOL 1985;151:632-5.)

Key words: Nutrition, cervix, cancer, vitamins

More than 200 years have elapsed since initial clinical studies suggested a relationship between scurvy and vitamin C deficiency.[1] During the ensuing years other clinical syndromes, particularly those associated with severe vitamin deficiencies, have been elucidated.[2] In general, vitamin deficiencies are considered evidence of malnutrition as the requirements for these nonmineral nutrients are usually low and most frequently occur as the result of concomitant disease such as malabsorption syndrome, alcoholism, liver disease, or food faddism.[2] However, these organic compounds serve as important catalytic cofactors in a number of biologic functions, and vitamin deficiences may prove to have an important impact in other aspects of preventive medicine.

Although the exact role of vitamins in clinical medicine is evolving, attention has been directed to specific vitamins in their role in promoting wound healing (vitamin C)[3] or the prevention of osteomalacia (vitamin D).[2] Most recently, considerable interest has been generated in the ability of vitamins to promote or prevent carcinogenesis.[4]

The increased incidence of cervical carcinoma in women of lower socioeconomic status coupled with the nutritional stress of multiple pregnancies in a situation of limited nutrient availability may result in vitamin as well as other nutritional deficits.

Unfortunately, few reports pertaining to vitamin deficiency in these patients are available. The purpose of this prospective evaluation was to determine the frequency of vitamin deficiencies in patients with untreated cervical cancer.

Methods and material

Between August, 1981, and December, 1982, 78 patients with untreated cancer of the uterine cervix who were admitted to the Gynecologic Oncology service at the University of Alabama at Birmingham underwent a prospective vitamin assessment. The characteristics of this population have been previously reported.[5] This evaluation was initially performed within 24 hours of admission and was repeated on the fifth postoperative day.

Vitamins studied included serum and red blood cell folate (*Lactobacillus casei* assay), vitamin A (fluorometric assay), β-carotene (colorimetric assay), vitamin B_{12} (Corning's Immo phase), vitamin C (chemical assay), thiamine (erythrocyte transketolase activity coefficient), riboflavin (erythrocyte glutathione reductase activity coefficient), and pyridoxine (erythrocyte aspartate aminotransferase activity coefficient).* Laboratory values found in the study were compared to predetermined normal values as defined by the evaluation of 240 healthy control patients at the Nutrition Diagnostic Laboratory.

Evidence of recent myocardial infarction, cardiac failure, significant airway disease, renal insufficiency,

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*More detailed information concerning these procedures is available upon request.

Table I. Vitamin assessment prior to operation

Vitamin	*Standard*	*Admission*	*% Below standard*	*Comparison with control values*
Folate (ng/ml)				
Plasma	2-10	4.4 ± 2.6 (1-16)	9	$p < 0.005$
Red blood cell	140-369	253 ± 107 (56-547)	12	NA
β-Carotene (μg/100 ml)	79-233	101 ± 59 (16-313)	38	$p < 0.0005$
Vitamin A (μg/100 ml)	25-70	46 ± 13 (18-90)	6	NS
Vitamin B_{12} (mg/100 ml)	205-700	427 ± 213 (135-1054)	9	NS
Vitamin C (mg/100 ml)	0.3-1.5	0.5 ± 0.4 (0.1-1.7)	33	$p < 0.0005$
Thiamine (A/C*)	>1.23	1.1 ± 0.1 (1-1.3)	9	NS
Riboflavin (A/C†)	>1.67	1.3 ± 0.2 (1-1.8)	4	$p < 0.08$
Pyridoxine (A/C‡)	>1.89	1.6 ± 0.3 (1-2.3)	14	NS

Values in parentheses are ranges.
*Erythrocyte transketolase activity coefficient.
†Erythrocyte glutathione reductase activity coefficient.
‡Erythrocyte aspartate aminotransferase activity coefficient.

Table II. Postoperative vitamin assessment

Vitamin	*Standard*	*% Decreased*	*% Abnormal*	
			Admission	*5 Days after operation*
Folate (ng/ml)				
Plasma	2-10	6	9	6
Red blood cell	140-369	0	12	8
β-Carotene (μg/100 ml)	79-233	60	38	76
Vitamin A (μg/100 ml)	25-70	20	6	24
Vitamin B_{12} (mg/100 ml)	205-700	20	9	17
Vitamin C (mg/100 ml)	0.3-1.5	15	33	13
Thiamine (A/C*)	>1.23	13	9	6
Riboflavin (A/C†)	>1.67	0	4	0
Pyridoxine (A/C‡)	>1.89	0	14	12

*Erythrocyte transketolase activity coefficient.
†Erythrocyte glutathione reductase activity coefficient.
‡Erythrocyte aspartate aminotransferase activity coefficient.

hepatic disease, or endocrinopathy eliminated patients from the study.

Data management was facilitated by computerization.

Results

Individual abnormal vitamin levels were present in 4% to 38% of patients (Table I). At least 67% of patients had one abnormal value, while 18% had three or more abnormal values. Only riboflavin levels decreased with age ($\chi^2: p < 0.04$), although the trend suggested that serum levels of β-carotene and vitamin A were lower in older individuals. Serum folate levels were abnormal in 30.8% of patients with advanced disease (Stage III or Stage IV) and demonstrated a significant ($\chi^2: p < 0.005$) stage-related decrease. β-Carotene levels, abnormally low in 69% of patients with advanced disease, was also significantly stage related ($\chi^2: p < 0.006$). Abnormal levels of serum albumin correlated with abnormal plasma levels of folate ($p < 0.03$), vitamin A ($p < 0.04$), and β-carotene ($p < 0.006$).

Mean admission values for plasma folate ($p < 0.005$), β-carotene ($p < 0.005$), and vitamin C were significantly lower than the mean established control value. Specific vitamin levels did not correlate with the diagnosis of protein-calorie malnutrition; however, hypoalbuminemia was correlated with low levels of β-carotene ($p < 0.004$) and vitamin B_{12} ($p < 0.006$). Low levels of vitamin C ($p = 0.013$) and folate ($p = 0.004$) correlated with low total iron-binding capacity. Postoperative values tended to decrease dramatically (Table II).

Comment

Although the role of specific vitamins in cancer therapy and prevention is currently emerging, abnormally low levels of folate, vitamin A, and vitamin C have been reported in as many as 45% of cancer patients with protein-calorie malnutrition.[7] Additionally, deficiencies in thiamine as well as other vitamins have been reported in patients with early or advanced malignant disease.[4, 7]

Recent attention has focused on the ability of vitamin A and related compounds (retinoids and carotenoids) to prevent the expression of malignant cellular behavior as well as to cause regression of established squamous cancer.[8] Patients with squamous cancer of the esophagus[9] or oral cavity[10] frequently have abnormal levels of vitamin A. However, some contradictory reports fail to confirm the presence of a low serum level of antioxidant vitamin A as a cancer risk.[11]

Deficiency of vitamin A is associated with an increase in cervical metaplasia[4] and the adverse effect of vitamin A or carotene deficiency on cell differentiation and growth may potentiate the effects of a local carcinogen or allow more rapid proliferation of malignant cells. Several reports have appeared with conflicting information concerning vitamin A levels in patients with premalignant cervical disease.[12, 13] At least one report suggests that the incidence of cervical cancer was inversely related to the intake of the precursor β-carotene but not preformed vitamin A.[8] This report represents the initial information on serum vitamin levels in a large group of patients with invasive cervical cancer. Six percent of patients with cervical cancer have vitamin A levels that are below normal, while 38% have stage-related abnormal levels of the precursor β-carotene. The latter mean values are significantly lower than control values. While low levels of β-carotene and vitamin A may be secondary to liver disease or liver storage abnormalities,[14] this study eliminated patients with apparent liver disease and these findings imply a general deficiency of vitamin A and its precursor β-carotene associated with cervical cancer. While blood levels may not equal tissue levels, the large percentage of abnormal laboratory values compared with established normal levels, combined with the reported antineoplastic effects of this vitamin and its related compounds, suggests the need for further evaluation of the role of this vitamin and its precursors in the prevention and therapy of cervical cancer.

Vitamin C may serve as an electron donor or acceptor, which may potentiate its role in wound healing, provide a protective function in maintaining normal epithelium, or potentially enhance or inhibit the activity of cancer-causing agents or carcinogens. Possible mechanisms of carcinogenic inhibition include alteration of the carcinogen's structure and competitive inhibition or prevention of carcinogen access to key target tissue by an increase in membrane stability. One recent report suggested a significant decrease in vitamin C intake in patients with cervical dysplasia[15] and implicated inadequate vitamin C intake as an independent risk factor for the development of premalignant cervical disease. Deficient levels of vitamin C in 33% of patients and mean levels that are lower in patients with invasive cervical cancer than in control subjects both support the findings of the previous investigation and require further investigation of this vitamin that blocks nitrosamine formation.[16]

Folic acid is thought to play an important role in the expression of the effects of steroid hormones on target tissues. The benefits of folic acid therapy in patients with cervical dysplasia, particularly those using oral contraceptives, have been reported.[17] The finding of abnormal plasma folate levels in 9% and abnormal red blood cell (representing tissue levels) folate in 12% of patients indicates that plasma or tissue levels of folate are less frequently abnormal than are levels of other vitamins. However, mean values are significantly less than laboratory control levels and the role of folic acid in the etiology of this malignancy should undergo further investigation.

It would appear patients with cervical cancer rarely exhibit abnormal levels of other vitamins and, unless other evidence surfaces, further evaluation of the role of vitamins in cervical cancer should be limited to the effects of those vitamins found to have a significant incidence of abnormalities. In addition, the lack of correlation of abnormal vitamin levels with other parameters of protein-calorie malnutrition[5] suggests the lack of a role for the routine use of vitamin screens in the initial nutritional evaluation of patients with cervical cancer unless the patient is nutritionally deficient or has a particular problem such as previous gastric (lack of intrinsic factor) or ileal (vitamin B_{12}) resection that predisposes her to a vitamin deficiency.

The demonstrated alterations of vitamin levels following operation make it imperative that future investigations into the role of vitamins in the etiology or treatment of cervical cancer monitor pretreatment values.

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Induction of ovulation with purified urinary follicle-stimulating hormone in patients with polycystic ovarian syndrome

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Purified urinary follicle-stimulating hormone was used to induce ovulation in 18 patients with polycystic ovarian syndrome. Each ampule contained 75 IU of follicle-stimulating hormone and <0.11 IU of luteinizing hormone. Initial doses were 150 to 225 IU/day, later increased to a maximum of 375 IU, according to daily clinical controls and estradiol values. After 12 to 16 days, follicle-stimulating hormone treatment was suspended. Within 36 to 48 hours each patient received 5000 or 10,000 IU of human chorionic gonadotropin, rarely more. Ovulation occurred in 39 of 43 treatment cycles and hyperstimulation in nine. Seven patients had normal pregnancies with viable fetuses, including one pair of twins. Two had abortions. Analysis of the endocrine situation during therapy does not permit either pregnancy or hyperstimulation to be predicted. However, hyperstimulation is frequently accompanied by endogenous luteinizing hormone peaks and greater estradiol increases during the final phase of induction. Purified follicle-stimulating hormone has thus demonstrated its validity in inducing ovulation in patients with polycystic ovarian syndrome, apparently with equal or lower risks of hyperstimulation than with other gonadotropin preparations. (AM J OBSTET GYNECOL 1985;151:635-40.)

Key words: Ovulation induction, purified follicle-stimulating hormone

The pharmacologic induction of ovulation in patients with polycystic ovarian syndrome depends almost exclusively on centrally acting drugs such as clomiphene or other antiestrogens.[1]

The high levels of endogenous luteinizing hormone in patients with polycystic ovarian syndrome increase the risk of hyperstimulation associated with use of the gonadotropins currently available.[2-8] Cases of hyperstimulation can be reduced by careful monitoring[9] or by the use of a preparation containing for the most part follicle-stimulating hormone.[10-14] For this reason we selected patients with polycystic ovarian syndrome, diagnosed by repeated hormone assays according to previous authors[3, 15] and confirmed by laparoscopy.[16] These patients were treated with a new gonadotropin prep-

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Table I. Data for the case studies

	No.	%
Treated patients	18	
Cycles of therapy	43	
Ovulatory cycles	39	
Ovulations without pregnancies	21	
Hyperstimulations	9	
Pregnancies	9	
Abortions	2	22
Deliveries	7*	78

*One bigeminal pregnancy at term and one bigeminal pregnancy with reabsorption of one twin and normal spontaneous delivery of a live and viable fetus. Therefore total number of viable fetuses was 8.

aration obtained from the urine of menopausal women, supplied by the Istituto Farmacologico Serono of Rome and containing 75 IU of follicle-stimulating hormone and <0.11 IU of luteinizing hormone.

The aim of our study was to verify the efficacy of the preparation in inducing ovulation and to investigate whether its therapeutic effect was greater than that of other available gonadotropins. Finally, we wish to establish endocrine criteria for the selection of patients in the hope of predicting their response to the drug.

Material and methods

Eighteen patients between 25 and 36 years of age with polycystic ovarian syndrome and desirous of having children were treated with purified urinary follicle-stimulating hormone. Fourteen patients had previously been treated with clomiphene (50 or 100 mg/day for 5 to 10 days) plus human chorionic gonadotropin. None had achieved pregnancy. Three patients had been treated with cycles of human menopausal gonadotropin plus human chorionic gonadotropin, which had resulted in hyperstimulation (Lunenfeld grade II)[5] in spite of careful monitoring (see below). For these three patients the treatment protocol followed for human menopausal gonadotropin was imitated during the first cycle of treatment with purified follicle-stimulating hormone. A total of 43 treatment cycles were administered, with the use (except in the three cycles just mentioned) of personalized protocols in order to achieve ovulation within 12 to 16 days. Treatment was initiated with 150 to 225 IU of follicle-stimulating hormone daily and increased in accordance with the ovarian response, which was monitored daily by means of clinical examination, basal body temperature, cervical score, and plasma estradiol levels. The maximum daily dose of follicle-stimulating hormone administered was 375 IU. Human chorionic gonadotropin was given in a single intramuscular injection of doses of between 5000 and 10,000 IU to all patients, generally 36 to 48 hours after suspension of treatment with follicle-stimulating hormone. Five cycles were treated with 15,000 IU of human chorionic gonadotropin in two doses (10,000 and 5000) on two consecutive days, while one patient, whose estradiol value was below 500 pg/ml on the day follicle-stimulating hormone was suspended, received 20,000 IU of human chorionic gonadotropin in three doses over 3 days (10,000 + 5000 + 5000). Serum samples were collected daily and stored at −20° C. Follicle-stimulating hormone, luteinizing hormone, prolactin, progesterone, estrone, testosterone, and androstenedione were all assayed. All assays for a single patient were carried out simultaneously with the use of radioimmunoassay kits (Biodata, Milan, Italy).

Results

Table I shows the data from our survey. Forty-three treatment cycles were administered and ovulation was achieved in 39 (91%) cycles and mild hyperstimulation (Lunenfeld II) in 9 (23%) ovulatory cycles. There were nine pregnancies (50% of patients), two of which were interrupted spontaneously at weeks 8 and 21. Two of the seven normal pregnancies were bigeminal: one, after early reabsorption of one fetus (echographically documented), concluded with the birth of a term fetus. All eight fetuses at birth appeared healthy, with normal infant weights. We divided the ovulatory cycles into 3 groups for the purposes of analyzing our results: ovulatory cycles not resulting in pregnancy, cycles resulting in pregnancy, and cycles resulting in hyperstimulation. We included in the first group those cycles in which the estradiol values were ≤2000 pg/ml on the day following suspension of the treatment and following which no ovarian cysts developed.

The follicle-stimulating hormone profile in Fig. 1 shows no significant variations in the different groups under consideration; differences may, however, be noted with regard to luteinizing hormone. The patients who conceived show constantly low values of luteinizing hormone in the first phase of stimulation. The ovulatory but nonpregnant patients show the same pattern but with greater standard deviation, which becomes excessive during the final days of treatment, thus manifesting the appearance of endogenous luteinizing hormone peaks. The hyperstimulated patients show more numerous and pronounced endogenous luteinizing hormone peaks during and after stimulation with purified follicle-stimulating hormone. The presence of an endogenous luteinizing hormone peak is not constant in all the cycles of a single patient. When it is present, the treatment frequently results in hyperstimulation, whereas when it is absent, pregnancy is more likely to result. Endogenous luteinizing hormone peaks were in fact present in 48% (10/21) of ovulatory cycles without pregnancy, in 56% (5/9) of hyperstimulated patients, and in 11% (1/9) of ovulatory cycles followed by pregnancy.

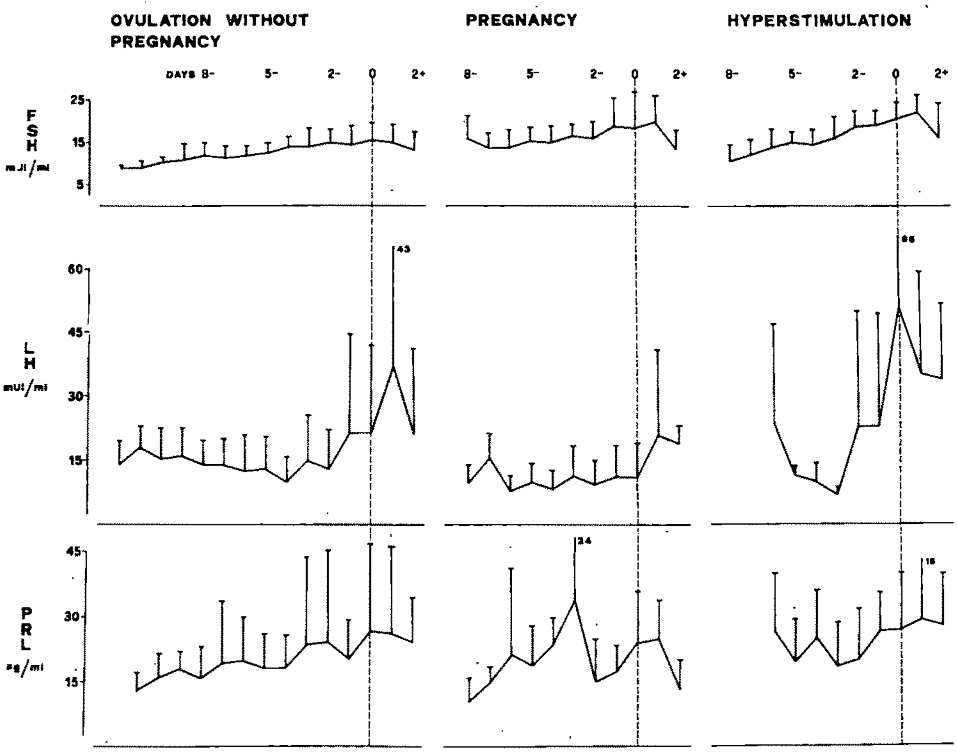

Fig. 1. Follicle-stimulating hormone *(FSH)*, luteinizing hormone *(LH)*, and prolactin *(PRL)* values in the three groups of patients. The patterns of follicle-stimulating hormone are similar in all groups. The luteinizing hormone levels are higher in the nonpregnant patients and especially in those with hyperstimulation (probably because of the presence of high basal values or endogenous peaks). The prolactin pattern is always the same in the three groups of patients. Each point corresponds to a number of hormone doses equal to or greater than 16 for ovulation without pregnancy and equal to or greater than six for hyperstimulations and pregnancies.

The prolactin pattern (Fig. 1) did not differ much from one group of patients to another. Individual values did, however, oscillate, tending to be higher in the hyperstimulated patients.

Fig. 2 shows the plasma estradiol values. During stimulation there were no significant differences between the different groups; in fact, the profile seems identical, although the hyperstimulated women reached higher values on the last day of follicle-stimulating hormone administration. These average values were 531 ± 215 pg/ml for the nonpregnant patients, 552 ± 259 pg/ml for the pregnant women, and 1145 ± 835 pg/ml for the hyperstimulated patients. The progesterone increase precedes the end of follicle-stimulating hormone treatment and the administration of human chorionic gonadotropin in the hyperstimulated and ovulatory cycles without pregnancy. This early progesterone increase could be the result of endogenous peaks of luteinizing hormone, which, as we have seen, are more frequent in these two groups. The association between early progesterone increase and endogenous luteinizing hormone peaks is evident from analysis of the single cases. Endogenous luteinizing hormone peaks were accompanied by progesterone increases in 30% of ovulatory cycles without pregnancy (2/5). In cycles resulting in hyperstimulation this figure is 40% (4/10). We found only one luteinizing hormone peak not followed by an increase in progesterone in the treatment cycles followed by pregnancy. We never found a progesterone increase without a luteinizing hormone peak.

Fig. 3 gives the profile of estradiol for the three patients to whom we administered first a cycle of stimulation with human menopausal gonadotropin and later a cycle of follicle-stimulating hormone at the same dosage. The pattern of estradiol increase shows no difference between the two types of stimulation.

Fig. 4 shows the marked increase in the standard deviation for estrone in the nonpregnant patients after suspension of the therapy and even earlier in the hyperstimulated patients. The testosterone and androstenedione values, shown in Fig. 4, were unchanged by the therapy in the different groups of patients.

Comment

Patients with polycystic ovarian syndrome present oligoamenorrhea and anovulation. The endocrine profile is marked by persistently high levels of luteinizing hormone with normal or low follicle-stimulating hor-

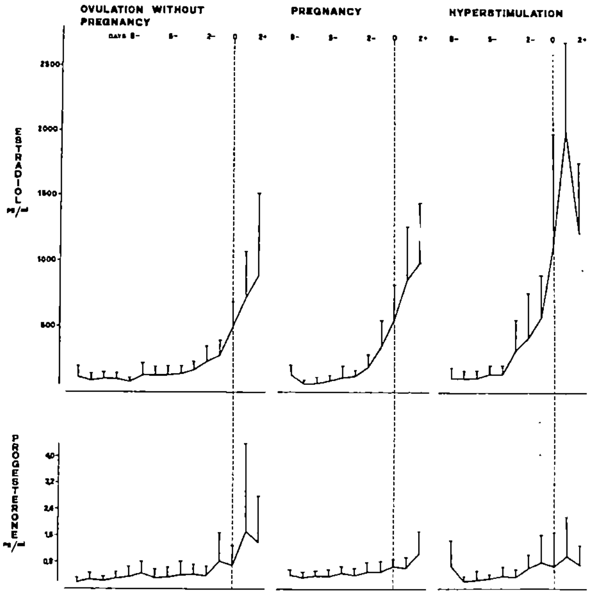

Fig. 2. *Estradiol* and *progesterone* levels in the three groups of patients. In the final phase of stimulation estradiol and progesterone levels tend to be higher in the hyperstimulated patients.

mone and high levels of androgens and estrone. The excess amount of luteinizing hormone stimulates ovarian production of androgens, which are converted into estrogens; these increase hypophyseal sensitivity to luteinizing hormone releasing factor, with consequent liberation of more luteinizing hormone. The comparative deficiency of follicle-stimulating hormone has been attributed either to an inhibitory effect of the intraovarian androgens or to the high levels of inhibin produced by the numerous follicles present in the ovaries of these patients.[17-19]

The induction of ovulation by pharmacologic means in these patients uses centrally acting drugs, such as clomiphene, which, by interrupting the estrogen feedback, restores a normal luteinizing hormone cycle. The use of the common human gonadotropin preparations in patients who do not respond to clomiphene treatment can be associated with a high percentage of hyperstimulation, because of the presence in the preparations of large quantities of luteinizing hormone. Careful monitoring during this therapy can mitigate this problem.

It has been shown that the use of purified follicle-stimulating hormone from human pituitaries is unable to induce follicular maturation in hypogonadotropic patients, since adequate levels of luteinizing hormone must be present.[12, 20, 21] The use of preparations of purified follicle-stimulating hormone for patients with polycystic ovarian syndrome can, on the other hand, give satisfactory results, given the high levels of endogenous luteinizing hormone present in this type of functional condition.

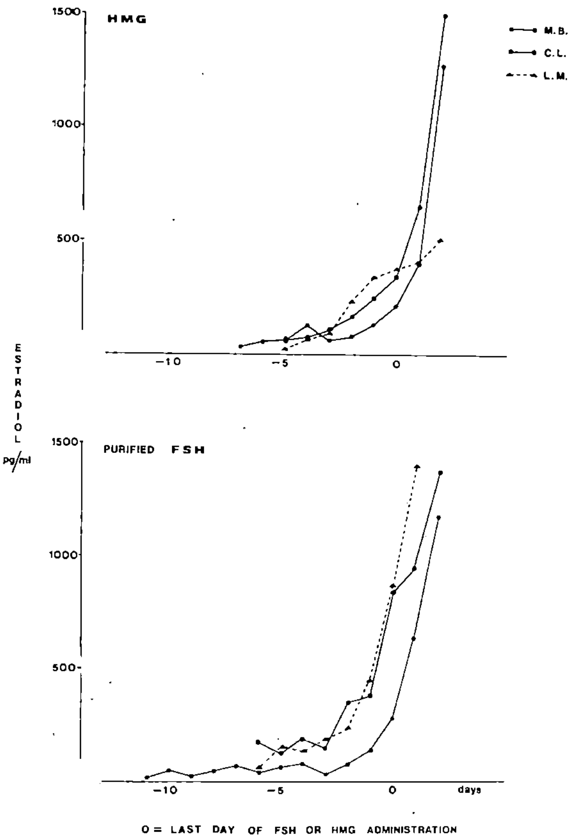

Fig. 3. 17β-Estradiol values in the three patients who first received a cycle of human menopausal gonadotropin *(HMG)* and later a cycle of follicle-stimulating hormone *(FSH)* at the same dosage. The pattern of *estradiol* increase shows no difference between the two types of stimulation.

In 1972 Berger et al.[12] reported the efficacy of a preparation of purified pituitary follicle-stimulating hormone in inducing follicular maturation in patients with polycystic ovarian syndrome. In 1977 Raj et al.[13] achieved 77% ovulation in 18 cycles of treatment with purified pituitary follicle-stimulating hormone in 10 patients with polycystic ovarian syndrome, with a 27% incidence of moderate hyperstimulation.

In 1982 Kamrava et al.[14] achieved two pregnancies in two patients with polycystic ovarian syndrome who were treated with purified pituitary follicle-stimulating hormone without the addition of human chorionic gonadotropin.

Our own data confirm the efficacy in women with polycystic ovarian syndrome of treatment with purified follicle-stimulating hormone extracted from the urine of menopausal women and virtually lacking in luteinizing hormone. In fact, this efficacy is emphasized by the following results: 91% of treatment cycles were ovulatory and nine cycles, equal to 50% of the patients, resulted in pregnancy. Because of the low number of hyperstimulations (23%), these results seem to be as valid as those obtained by us in patients with hypopituitarism after treatment with human menopausal gonadotropin plus human chorionic gonadotropin.[4,6] The incidence of abortions was 22%, as in hypogonadotropic patients stimulated with human menopausal gonadotropin.[22] These results are more optimistic than those obtained by Venturoli et al.,[23] who did not get any pregnancy at term in five patients affected with polycystic ovarian syndrome and treated with Pergonal and subsequently with follicle-stimulating hormone.

The estradiol levels on suspension of treatment were not reliable parameters for predicting the results of stimulation. For example, average estradiol values in the nonpregnant and pregnant patients were fairly similar on the suspension of stimulation (531 ± 215 and 552 ± 259 pg/ml). It is probable that in many women of the former group this value is obtained by the simultaneous maturation of several follicles, as our occasional ultrasound examinations suggested. In the hyperstimulated women, on the other hand, the average estradiol values on suspension of follicle-stimulating hormone were decidedly greater (1145 ± 835 pg/ml), but the difference was not statistically significant. In some of these cases this would have been avoided by interrupting the administration of follicle-stimulating hormone earlier or by not administering human chorionic gonadotropin. In many cases, however, it was the spontaneous appearance of endogenous luteinizing hormone peaks that caused hyperstimulation.

Maybe the basal androgenic situation of the ovary, and certainly the reactivity of the hypothalamic-pituitary axis in terms of luteinizing hormone production, could be a decisive element for the outcome of therapy.

When this activation fails to assume any physiologic aspect, whether for reasons intrinsic to the ovary or because of high basal levels of androstenedione, the outcome of stimulation is jeopardized.

Another decisive factor influencing the outcome of stimulation is the occurrence of an endogenous luteinizing hormone peak. The increase in estradiol induced by follicle-stimulating hormone often causes a positive estrogen feedback for luteinizing hormone in these patients. This endogenous peak, especially when induced by estrogens from the simultaneous maturation of more than one follicle, may occur when the ovary is not ready for ovulation and may thus provoke follicular luteinization or ineffective ovulation. In other situations this endogenous peak, when added to exogenous administration of human chorionic gonadotropin, could induce hyperstimulation. In our patients, in whom the assays of luteinizing hormone were carried out later, we often found endogenous peaks of luteinizing hormone, both in hyperstimulated and normostimulated but nonpregnant patients. This phenomenon may be one of the possible causes for the lack of pregnancy in the latter group.

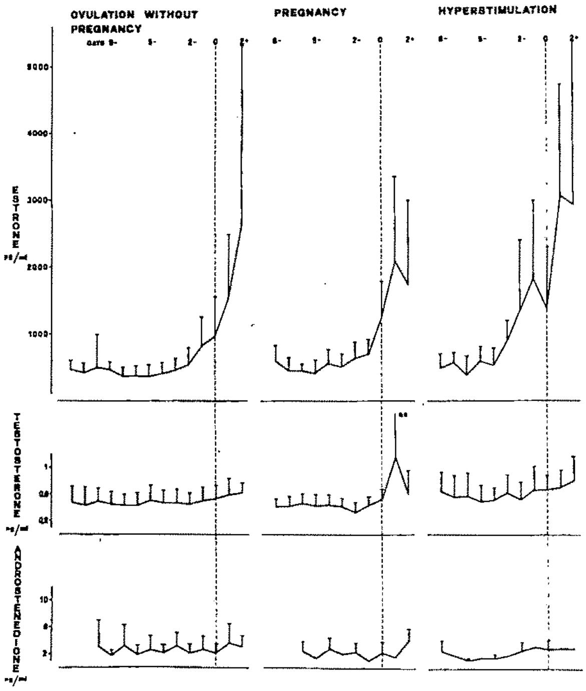

Fig. 4. *Estrone, testosterone,* and *androstenedione* patterns in the three groups. Estrone is higher in the periovulatory phase in the hyperstimulated and ovulatory but nonpregnant women. Testosterone and androstenedione are not modified by the therapy.

Purified follicle-stimulating hormone was shown in our experience to be capable of producing normal follicular maturation followed by a normal pregnancy in women affected with polycystic ovarian syndrome. The percentage of pregnancies we obtained was high. The number and severity of cases of hyperstimulation were similar to those obtained with other gonadotropic preparations.

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Thyroid function in epileptic mothers and their infants at birth

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It has been suggested that patients receiving anticonvulsant therapy have depressed thyroid function. Thyroid function was studied in 16 pregnant epileptic women who were receiving various anticonvulsants; 20 nonepileptic pregnant women served as controls. Maternal and umbilical cord blood was collected at delivery and serum thyrotropin, total thyroxine, triiodothyronine, triiodothyronine resin uptake, and free thyroxine levels were measured. The free thyroxine index was calculated from the thyroxine and triiodothyronine resin uptake data. There were no significant differences in any of the maternal parameters. In cord serum, the thyroxine level was significantly lower ($p < 0.001$) in the infants of the epileptic mothers. The triiodothyronine resin uptake was slightly increased in the epileptic group ($p < 0.05$) so that the free thyroxine index largely compensated for this. The thyrotropin, free thyroxine, and triiodothyronine levels did not differ between the two groups. Thus the low thyroxine values in cord blood of infants of epileptic mothers receiving anticonvulsants probably reflect an alteration in protein binding rather than a true alteration in thyroid function. (AM J OBSTET GYNECOL 1985;151:641-4.)

Key words: Thyroid, anticonvulsant, epilepsy, pregnancy

Antiepileptic drug therapy is usually necessary to control seizures in epileptic women in pregnancy. However, anticonvulsants cross the placental barrier[1] and may be teratogenic to the fetus. The main concerns have been with the severe malformations associated with the fetal hydantoin syndrome.[2] Studies in pregnant mice given high doses of antiepileptic drugs have shown that these drugs decrease fetal body weight[3] and brain weight,[4] and it is suspected that the same delay in fetal development and fetal growth occurs in man.

Relatively little information is available concerning the effects of antiepileptic drugs on hormones during pregnancy. Several studies have shown that antiepileptic drugs have an effect on thyroid function in nonpregnant women.[5-8] Phenytoin (diphenylhydantoin) has been shown to decrease serum protein-bound iodine, and it has been suggested that it displaces thyroxine from its binding sites on thyroxine-binding globulin.[9, 10] A recent study with anticonvulsants in pregnancy has revealed that these drugs depress serum thyroxine levels in infants and mothers and it has been suggested that such changes might be responsible for the growth retardation of the fetal head characteristic of the offspring exposed to anticonvulsants in utero.[11]

The purpose of this study was to determine the effects of anticonvulsants on serum thyroid hormone levels in pregnant epileptic women and their children at birth.

Patients and methods

Thyroid function was studied in 16 pregnant epileptic women attending the Montreal Neurological Institute Seizure Clinic. They were receiving a variety of anticonvulsant medications (phenytoin, phenobarbital, carbamazepine, ethosuximide, valproic acid, and primidone; either singly or in combination). Twenty nonepileptic pregnant women served as the controls. Umbilical cord blood from 14 of the epileptic patients and from 17 control patients was also collected. All samples were drawn into heparinized tubes at the time of delivery. Blood samples were centrifuged at 4° C and the serum was separated and stored at −20° C until assayed. Thyroxine, triiodothyronine, triiodothyronine resin uptake (a measure of binding to thyroxine-binding globulin), and thyroid-stimulating hormone were measured by the automated radioassay instrument ARIA II (Becton-Dickinson, Salt Lake City, Utah), and free thyroxine was measured with the Gammacoat free thyroxine radioimmunoassay kit (Clinical Assays, Cambridge, Massachusetts). The free thyroid index was calculated from the total thyroxine and triiodothyronine resin uptake data:

$$\text{Thyroxine} \times \frac{\text{triiodothyronine resin uptake of patient}}{\text{triiodothyronine resin uptake of mean for nonpregnant control subjects (30\%)}}$$

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Table I. Thyroid hormone levels in maternal blood

Hormone	*Control*	*Epileptic patients receiving phenytoin*	*p Value*	*All epileptic patients*	*p Value*
Thyrotropin (0-9 μU/ml*)					
Mean	1.34	1.25		1.33	
SD	±1.08	±0.83	NS	±0.76	NS
SE	±0.24	±0.26		±0.19	
n	20	10		16	
Total thyroxine (4-11 μg/dl)					
Mean	11.30	9.28	$0.1<p>0.05$	9.80	
SD	±2.78	±2.74		±2.51	NS
SE	±0.62	±0.87		±0.63	
Free thyroxine (0.7-1.8 ng/dl)					
Mean	0.57	0.56		0.57	
SD	±0.26	±0.18	NS	±0.17	NS
SE	±0.06	±0.06		±0.04	
Triiodothyronine (65-200 ng/dl)					
Mean	188.3	145.6		162.6	
SD	±38.6	±57.5	$p<0.05$	±54.5	NS
SE	±8.6	±18.2		±13.6	
Triiodothyronine resin uptake (25-35%)					
Mean	18.1	19.04		18.3	
SD	±3.6	±5.5	NS	±3.7	NS
SE	±0.82	±1.4		±0.93	
Free thyroxine index (4.9-9.8)					
Mean	6.50	5.68		5.88	
SD	±0.87	±1.36	NS	±1.31	NS
SE	±0.19	±0.43		±0.33	

NS = Not significant.

*Number in parentheses is normal range for nonpregnant adults.

Group means for epileptic and control subjects were compared with Student's *t* test.

Results

The determinations for thyroid hormone levels in the maternal sera are summarized in Table I. Compared to normal nonpregnant subjects, both epileptic and control groups exhibited high thyroxine and low triiodothyronine resin uptake (indicating increased thyroxine-binding globulin binding) with normal free thyroxine index, data in accord with many previous studies. The triiodothyronine levels were also high, presumably because of increased thyroxine-binding globulin binding, while thyrotropin levels were normal and free thyroxine levels were low.

Although the maternal mean levels of total thyroxine, triiodothyronine, and free thyroxine index in epileptic patients were lower than those of the control subjects, the differences just failed to reach significance ($0.05 < p < 0.1$).

In the cord sera (Table II) of epileptic and control patients compared to those of nonpregnant adults, thyrotropin levels were elevated above the normal range, while triiodothyronine levels were at the lower limit. The thyroxine levels in the control group were borderline high, with correspondingly low triiodothyronine resin uptake levels and normal free thyroxine index levels. The thyroxine levels in the epileptic group were in the midnormal range, with normal binding, and normal free thyroxine index and free thyroxine values.

When the cord sera of epileptic mothers were compared with those from control subjects, thyroxine levels from the epileptic group were significantly reduced ($p < 0.001$), binding was not elevated as in the control group ($p < 0.05$), and the free thyroxine index levels were lower ($p < 0.01$) although still in the normal midrange for nonpregnant adults. There were no differences in levels of thyrotropin, triiodothyronine, and free thyroxine.

When epileptic patients receiving phenytoin were analyzed separately, the values were little altered. The triiodothyronine levels in the phenytoin-treated group were lower than those of the maternal control group ($p < 0.05$) but still within the normal nonpregnant midrange.

Comment

The mechanisms of action of anticonvulsants on thyroid function are not entirely understood. In vitro, phenytoin competes weakly for thyroxine-binding sites on thyroxine-binding globulin.[9, 10] In vivo studies suggest that phenytoin alters the clearance and metabolism of thyroxine and possibly also of triiodothyronine.[5] In

Table II. Thyroid hormone levels in cord blood

Hormone	*Control*	*Epileptic patients receiving phenytoin*	*p Value*	*All epileptic patients*	*p Value*
Thyrotropin (μU/ml)					
Mean	7.95	8.03		7.34	
SD	±4.2	±5.7	NS	±4.6	NS
SE	±1.0	±1.9		±1.2	
n	17	9		14	
Total thyroxine (μg/dl)					
Mean	10.0	7.49	$p < 0.001$	7.88	
SD	±1.7	±1.1		±1.5	$p < 0.001$
SE	±0.42	±0.33		±0.39	
Free thyroxine (ng/dl)					
Mean	1.30	1.20		1.15	
SD	±0.18	±0.27	NS	±0.27	NS
SE	±0.04	±0.09		±0.07	
Triiodothyronine (ng/dl)					
Mean	70.3	60.3		60.6	
SD	±20.1	±12.9	NS	±12.6	NS
SE	±4.9	±4.3		±3.4	
Triiodothyronine resin uptake (%)					
Mean	20.9	23.8		23.4	
SD	±1.7	±5.6	$0.1 < p > 0.05$	±5.0	$p < 0.05$
SE	±0.4	±1.9		±1.3	
Free thyroxine index					
Mean	6.9	5.8		6.0	
SD	±0.85	±1.4	$p < 0.01$	±0.75	$p < 0.01$
SE	±0.21	±0.45		±0.20	

NS = Not significant.

non-pregnant epileptic adult subjects studied by Molholm-Hansen et al.,[5] serum thyroxine, triiodothyronine, free thyroxine index, and free thyroxine were reduced while thyrotropin and thyrotropin response to thyrotropin-releasing factor increased. Rootwelt et al.[7] and Yeo et al.[8] found that thyroxine, free thyroxine, free thyroxine index, triiodothyronine, free triiodothyronine, free triiodothyronine index, and reverse triiodothyronine were reduced while triiodothyronine resin uptake and thyrotropin were not affected.

In epileptic children, Fichsel and Knopfle[6] found that long-term therapy with valproate, phenytoin, primidone, carbamazepine, or combinations of these caused decreased thyroxine, free thyroxine, and triiodothyronine levels as in adults. However, thyrotropin tended to be decreased rather than increased in the short term, and thyrotropin response to thyrotropin-releasing factor was normal.

In pregnancy, thyroxine-binding globulin levels rise so that thyroxine and triiodothyronine are elevated and triiodothyronine resin uptake is decreased. Even when corrected for triiodothyronine resin uptake, the free thyroxine index is lower in the third trimester and the free thyroxine level is also lower than in nonpregnant subjects,[12] while thyrotropin is not altered. Cord serum levels at delivery are characterized by high thyroxine, low triiodothyronine resin uptake (high thyroxine-binding globulin), and increased free thyroxine levels.[14]

The only studies of the effects of anticonvulsants on thyroid function in pregnancy are those of Kaneko et al.,[11] who found lower thyroxine levels in maternal and cord sera of the epileptic group compared with the control group, and that of Luoma et al.,[13] who found that treating epileptic women late in pregnancy with phenobarbital lowered thyroxine and free thyroxine index. However, Kaneko et al. did not include any binding studies. Our results are in general agreement with those of Kaneko et al. for third-trimester maternal serum and for cord serum. However, the decrease in thyroxine in the maternal sera of our patients failed to reach significance while that in Kaneko's group was significant only at $p < 0.05$ in their comparable group at term, the second-trimester difference being greater. When corrected for differences in binding, the free thyroxine index values in both our control and epileptic subjects fell to the midnormal range for nonpregnant subjects. Consideration of subjects receiving phenytoin as a separate group did not alter the results except for triiodothyronine, and this was only marginally significant ($p < 0.05$).

The difference between the control and epileptic cord serum thyroxine value (7.9 μg/dl in epileptic group, versus 10.0 μg/dl in control group) was more marked in our study ($p < 0.001$) and was partly corrected by taking into consideration the decreased binding to thyroxine-binding globulin of the epileptic group relative to the control group so that the free thyroxine index remained slightly lower ($p < 0.001$) but was well within the euthyroid range (6.0 ± 0.45 in the epileptic group versus 6.9 ± 0.21 in the control group). No individual value fell below the lower limit of the normal range (4.9) in either group. There was no significant difference between the free thyroxine values of the two groups.

Values for free thyroxine did not differ between control and epileptic groups but were slightly lower than those found by others using clotted blood owing to the effect of heparin.[15]

Although anticonvulsants clearly lower thyroxine levels in cord serum, the thyrotropin is unaltered and the effect appears to be largely (free thyroxine index) or entirely (free thyroxine) explained by altered protein binding so that the infants of epileptic mothers do not have hypothyroid indices at the time of birth. Furthermore, since infants discovered to be hypothyroid by screening procedures at birth appear to suffer no sequelae of myxedema if treated within 5 months after birth,[16] it seems unlikely that decreased fetal thyroid function plays any role in the development of the fetal hydantoin syndrome.

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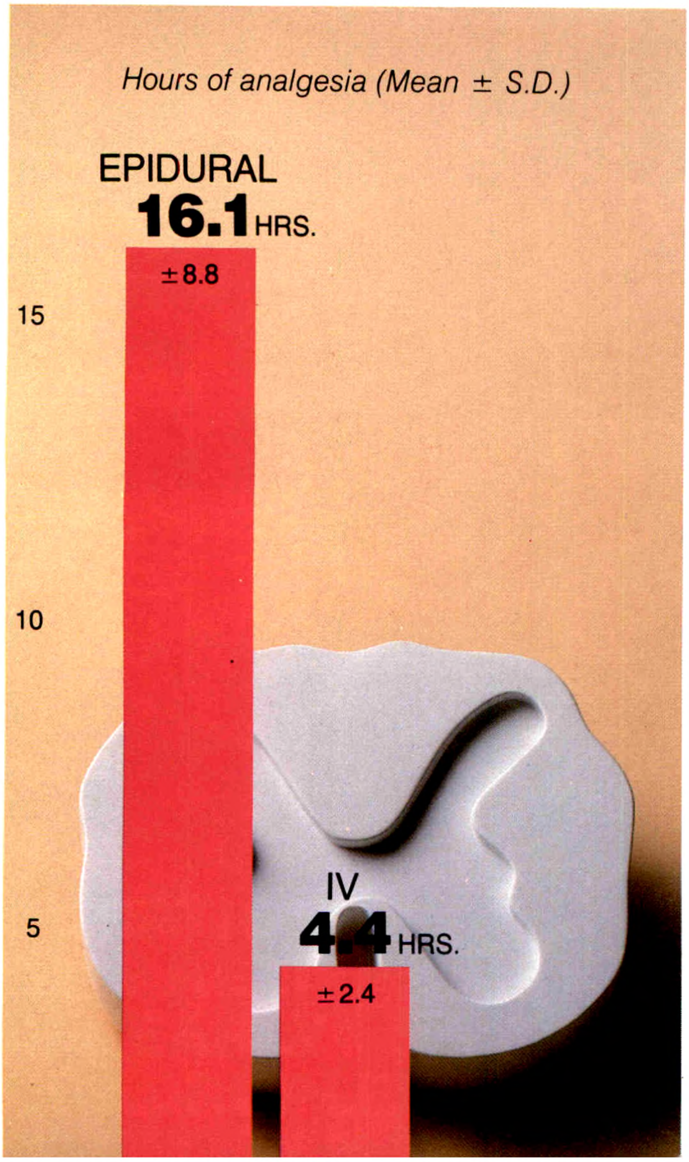

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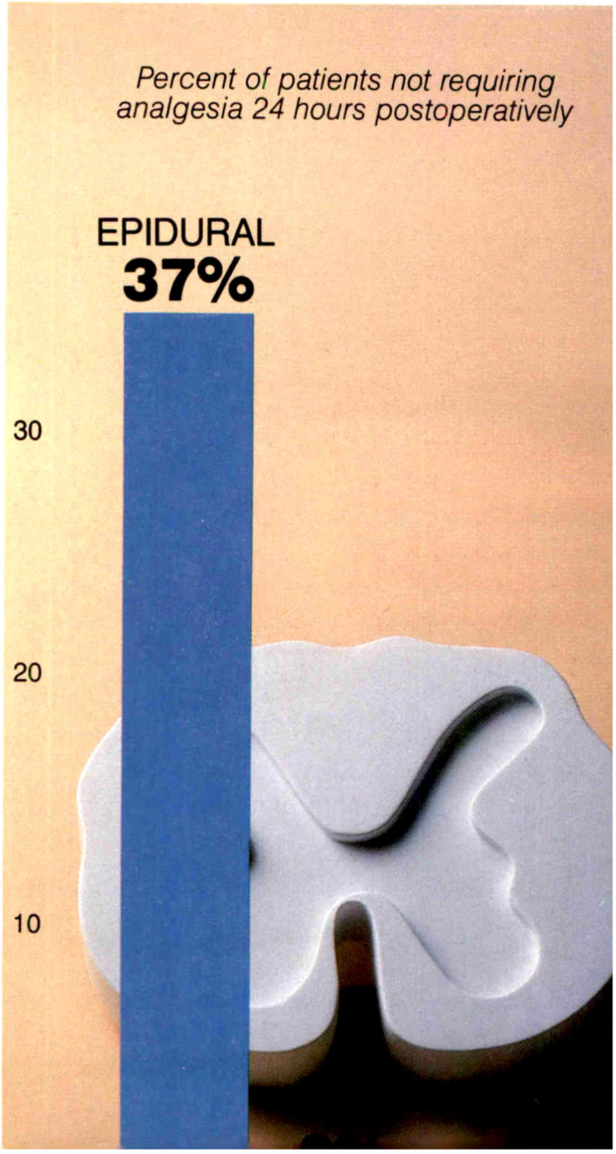

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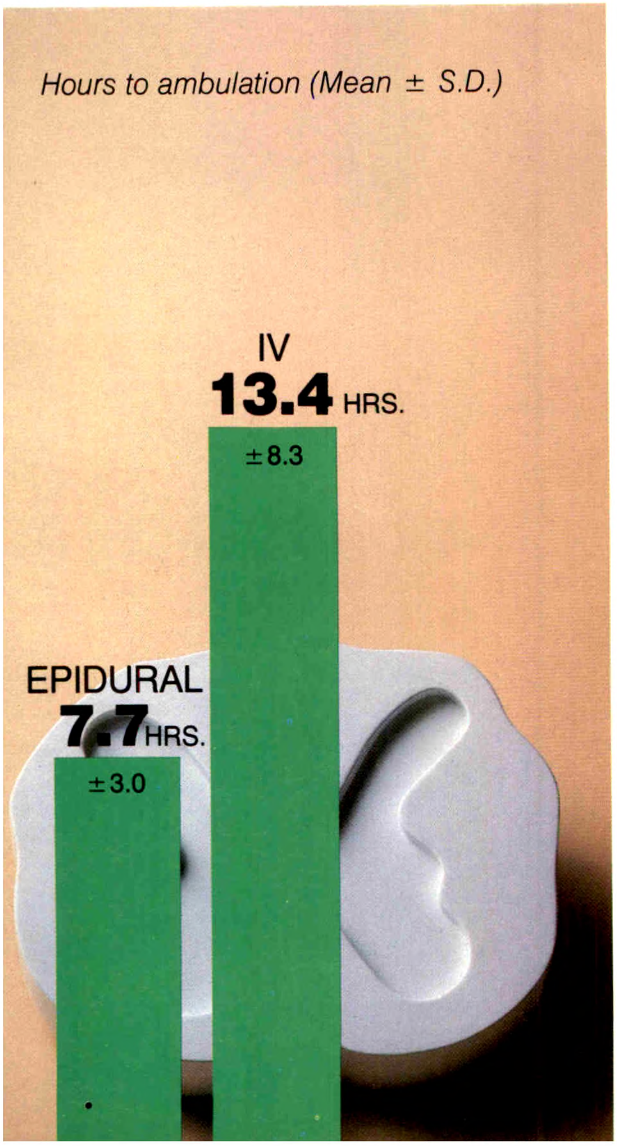

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*Cohen SE, Woods WA: Anesthesiology 58:500, 1983

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CONTRAINDICATIONS

DURAMORPH® PF is contraindicated in those medical conditions which would preclude the administration of opioids by the intravenous route—allergy to morphine or other opiates, acute bronchial asthma, upper airway obstruction.

Administration of morphine by the epidural or intrathecal route is contraindicated in the presence of infection at the injection site, anticoagulant therapy, bleeding diathesis, parenterally administered corticosteroids within a two week period or other concomitant drug therapy or medical condition which would contraindicate the technique of epidural or intrathecal analgesia.

WARNINGS

DURAMORPH® PF administration should be limited to use by those familiar with the management of respiratory depression, and in the case of epidural or intrathecal administration, familiar with the techniques and patient management problems associated with epidural or intrathecal drug administration. Because epidural administration has been associated with lessened potential for immediate or late adverse effects than intrathecal administration, the epidural route should be used whenever possible. Rapid intravenous administration may result in chest wall rigidity.

FACILITIES WHERE DURAMORPH® PF IS ADMINISTERED MUST BE EQUIPPED WITH RESUSCITATIVE EQUIPMENT, OXYGEN, NALOXONE INJECTION, AND OTHER RESUSCITATIVE DRUGS. WHEN THE EPIDURAL OR INTRATHECAL ROUTE OF ADMINISTRATION IS EMPLOYED, PATIENTS MUST BE OBSERVED IN A FULLY EQUIPPED AND STAFFED ENVIRONMENT FOR AT LEAST 24 HOURS.

SEVERE RESPIRATORY DEPRESSION UP TO 24 HOURS FOLLOWING EPIDURAL OR INTRATHECAL ADMINISTRATION HAS BEEN REPORTED.

Morphine sulfate may be habit forming. (See Drug Abuse and Dependence section.)

PRECAUTIONS

GENERAL

Preservative-free DURAMORPH® PF (Morphine Sulfate Injection, USP) should be administered with extreme caution in aged or debilitated patients, in the presence of increased intracranial/intraocular pressure and in patients with head injury. Pupillary changes (miosis) may obscure the course of intracranial pathology. Care is urged in patients who have a decreased respiratory reserve (e.g., emphysema, severe obesity, kyphoscoliosis).

Seizures may result from high doses. Patients with known seizure disorders should be carefully observed for evidence of morphine-induced seizure activity.

It is recommended that administration of DURAMORPH® PF by the epidural or intrathecal routes be limited to the lumbar area. Intrathecal use has been associated with a higher incidence of respiratory depression than epidural use.

Smooth muscle hypertonicity may result in biliary colic, difficulty in urination and possible urinary retention requiring catheterization. Consideration should be given to risks inherent in urethral catheterization, e.g., sepsis, when epidural or intrathecal administration is considered, especially in the perioperative period.

Elimination half-life may be prolonged in patients with reduced metabolic rates and with hepatic or renal dysfunction. Hence, care should be exercised in administering morphine in these conditions, particularly with repeated dosing.

Patients with reduced circulating blood volume, impaired myocardial function or on sympatholytic drugs should be observed carefully for orthostatic hypotension, particularly in transport.

Patients with chronic obstructive pulmonary disease and patients with acute asthmatic attack may develop acute respiratory failure with administration of morphine. Use in these patients should be reserved for those whose conditions require endotracheal intubation and respiratory support or control of ventilation.

DRUG INTERACTIONS

Depressant effects of morphine are potentiated by either concomitant administration or in the presence of other CNS depressants such as alcohol, sedatives, antihistaminics or psychotropic drugs (e.g., MAO inhibitors, phenothiazines, butyrophenones and tricyclic antidepressants). Premedication or intra-anesthetic use of neuroleptics with morphine may increase the risk of respiratory depression.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Studies of morphine sulfate in animals to evaluate the carcinogenic and mutagenic potential or the effect on fertility have not been conducted.

PREGNANCY

Teratogenic effects—Pregnancy Category C. Animal reproduction studies have not been conducted with morphine sulfate. It is also not known whether morphine sulfate can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Morphine sulfate should be given to a pregnant woman only if clearly needed.

Nonteratogenic effects. Infants born from mothers who have been taking morphine chronically may exhibit withdrawal symptoms.

LABOR AND DELIVERY

Intravenous morphine readily passes into the fetal circulation and may result in respiratory depression in the neonate. Naloxone and resuscitative equipment should be available for reversal of narcotic-induced respiratory depression in the neonate. In addition, intravenous morphine may reduce the strength, duration and frequency of uterine contraction resulting in prolonged labor.

Epidurally and intrathecally administered morphine readily passes into the fetal circulation and may result in respiratory depression of the neonate. Controlled clinical studies have shown that *epidural* administration has little or no effect on the relief of labor pain.

However, studies have suggested that in most cases 0.2 to 1 mg of morphine *intrathecally* provides adequate pain relief with little effect on the duration of first stage labor. The second stage labor, though, may be prolonged if the parturient is not encouraged to bear down. A continuous intravenous infusion of naloxone, 0.6 mg/hr, for 24 hours after intrathecal injection may be employed to reduce the incidence of potential side effects.

NURSING MOTHERS

Morphine is excreted in maternal milk. Effect on the nursing infant is not known.

PEDIATRIC USE

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

The most serious side effect is respiratory depression. Because of
delay in maximum CNS effect with intravenously administered
(30 min), rapid administration may result in overdosing. B
administration by the epidural or intrathecal route may resu
early respiratory depression due to direct venous redistributio
morphine to the respiratory centers in the brain. Late (up t
hours) onset of acute respiratory depression has been repo
with administration by the epidural or intrathecal route an
believed to be the result of rostral spread. Reports of respira
depression following intrathecal administration have been n
frequent, but the dosage used in most of these cases has b
considerably higher than that recommended. This depression
be severe and could require intervention (See Warnings and O
dosage sections). Even without clinical evidence of ventila
inadequacy, a diminished CO_2 ventilation response may be n
for up to 22 hours following epidural or intrathecal administrat

While low doses of intravenously administered morphine have l
effect on cardiovascular stability, high doses are excitatory, res
ing from sympathetic hyperactivity and increase in circula
catecholamines. Excitation of the central nervous system resul
in convulsions may accompany high doses of morphine gi
intravenously. Dysphoric reactions may occur and toxic psycho
have been reported.

Epidural or intrathecal administration is accompanied by a h
incidence of pruritus which is dose related but not confined to
of administration. Nausea and vomiting are frequently seer
patients following morphine administration. Urinary retent
which may persist for 10-20 hours following single epidura
intrathecal administration has been reported in approximately 9
of males. Incidence is somewhat lower in females. Patients r
require catheterization (see Precautions). Pruritus, naus
vomiting and urinary retention frequently can be alleviated by
intravenous administration of low doses of naloxone (0.2 mg)

Tolerance and dependence to chronically administered morphi
by whatever route, is known to occur (see Drug Abuse and Dep
dence section).

Miscellaneous side effects include constipation, headache, anxi
depression of cough reflex, interference with thermal regulat
and oliguria. Evidence of histamine release such as uticaria, whe
and/or local tissue irritation may occur.

In general, side effects are amenable to reversal by narcotic ant
onists. NALOXONE INJECTION AND RESUSCITATIVE EQUIPME
SHOULD BE IMMEDIATELY AVAILABLE FOR ADMINISTRATION
CASE OF LIFE-THREATENING OR INTOLERABLE SIDE EFFEC

DRUG ABUSE AND DEPENDENCE

Controlled Substance: Morphine sulfate is a Schedule II substa
under the Drug Enforcement Administration classification.

Abuse: Morphine has recognized abuse potential.

Dependence: Cerebral and spinal receptors may develop to
ance/dependence independently, as a function of local dosa
Care must be taken to avert withdrawal in those patients who h
been maintained on parenteral/oral narcotics when epidural
intrathecal administration is considered. Withdrawal may oc
following chronic epidural or intrathecal administration, as well
the development of tolerance to morphine by these routes. (
Nonteratogenic effects under Pregnancy.)

OVERDOSAGE

Overdosage is characterized by respiratory depression with
without concomitant CNS depression. Since respiratory arrest m
result either through direct depression of the respiratory center
as the result of hypoxia, primary attention should be given to t
establishment of adequate respiratory exchange through provisi
of a patent airway and institution of assisted or controlled venti
tion. The narcotic antagonist, naloxone, is a specific antido
Naloxone (usually 0.4 mg) should be administered intravenous
simultaneously with respiratory resuscitation. *As the duration*
effect of naloxone is considerably shorter than that of epidural
intrathecal morphine, repeated administration may be necessa
Patients should be closely observed for evidence of renarcotizatio
Note: Respiratory depression may be delayed in onset up to
hours following epidural or intrathecal administration. In pain
conditions, reversal of narcotic effect may result in acute onset
pain and release of catecholamines. Careful administration
naloxone may permit reversal of side effects without affecti
analgesia. Parenteral administration of narcotics in patients rece
ing epidural or intrathecal morphine may result in overdosage.

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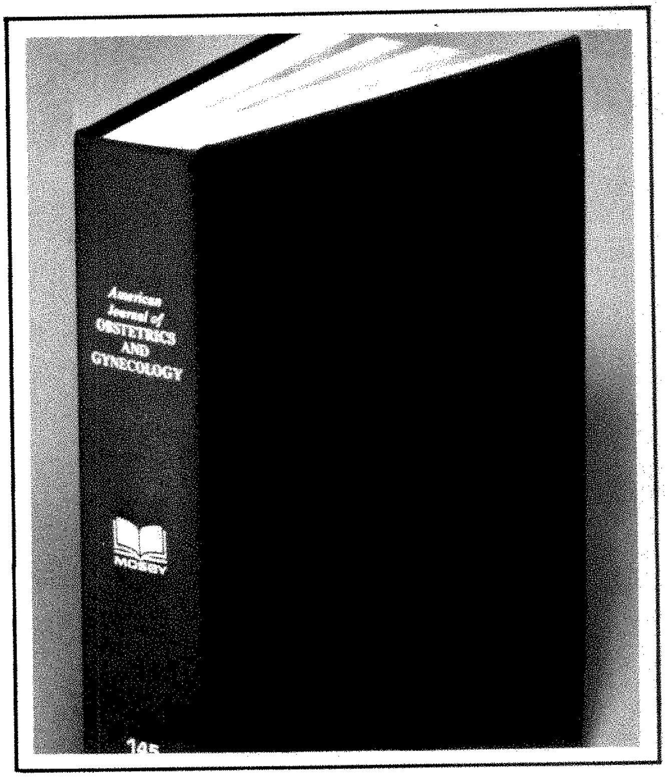

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[1] Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.

*Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.

†Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

‡Data on file for 22,489 total cycles, Wyeth Laboratories. **See important information on following page.**

IN BRIEF:

Indications and Usage—LO/OVRAL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Comparison of endometrial biopsy and peritoneal fluid cytologic testing with laparoscopy in the diagnosis of acute pelvic inflammatory disease

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Twenty-seven women with suspected acute pelvic inflammatory disease were studied by laparoscopy and endometrial biopsy. Overall, 67% (18 of 27) of the women had acute salpingitis at laparoscopy, 70% (19 of 27) had plasma cell endometritis, and 67% (10 of 15) had an inflammatory cytologic pattern of the peritoneal fluid. In comparison to laparoscopically detected salpingitis, endometritis detected with biopsy had a sensitivity of 89%, a specificity of 67%, a positive predictive value of 84%, and a false negative rate of 22% in the diagnosis of pelvic inflammatory disease. The corresponding figures for inflammation of the peritoneal fluid were 75%, 67%, 90%, and 25%. This study demonstrates that nonpuerperal endometritis is an entity associated with pelvic inflammatory disease, most likely representing an intermediate stage between cervicitis and salpingitis. Endometrial biopsy as an office procedure is a good alternative to laparoscopy in the diagnosis of acute pelvic inflammatory disease and may in fact detect early cases not yet visible at laparoscopy. (AM J OBSTET GYNECOL 1985;151:645-50.)

Key words: Pelvic inflammatory disease, endometrial biopsy, laparoscopy, peritoneal fluid cytology

Acute salpingitis is usually an infection ascending from the lower genital tract.[1, 2] Salpingitis is best diagnosed at laparoscopy.[2-7] However, endometritis as an early state of infection leading to acute salpingitis has not been thoroughly studied. Similarly, the cellular pattern of peritoneal fluid in acute salpingitis is largely unknown. Therefore, we designed a prospective study among women with suspected acute pelvic inflammatory disease to address other confirmatory tests for acute salpingitis, such as endometrial biopsy and peritoneal fluid cytologic results.

Material and methods

Study population. Consecutive women with suspected acute pelvic inflammatory disease who were admitted to the Department of Obstetrics and Gynecology, University Central Hospital, Tampere, Finland, were eligible for the study. Twenty-seven such women were asked to participate, and 26 of them agreed. The clinical criteria for the diagnosis of acute pelvic inflammatory disease that were used as the basis of inviting patients to participate were the history of low abdominal pain of less than 3 weeks' duration and the presence of cervical motion, uterine, and adnexal tenderness. Women who had received antibiotics, had undergone a gynecologic operation, or had any instrumentation of the upper genital tract in the prior month were excluded.

Patient evaluation. A standardized interview concerning menstrual history, contraceptive method, prior gynecologic diseases, and current symptoms was obtained from all women. All women underwent gynecologic examination, and the presence of any cervical motion tenderness or uterine and adnexal tenderness was determined by bimanual examination. The women were then scheduled for laparoscopy, which was performed within 20 hours. Antimicrobial therapy was started intravenously immediately following the laparoscopy.

Collection of specimens. During general anesthesia an unlubricated speculum was inserted into the vagina and the cervix was exposed. After the cervix was wiped with a large cotton-tipped swab, a sterile, disposable Vabra (Ferrosan Co., Copenhagen, Denmark) endometrial suction curette equipped with a 3 mm metallic tip was inserted transcervically. A single-strip endometrial biopsy specimen was successfully obtained in all cases. The sections of formalin-fixed and paraffin-embedded specimens were routinely stained according

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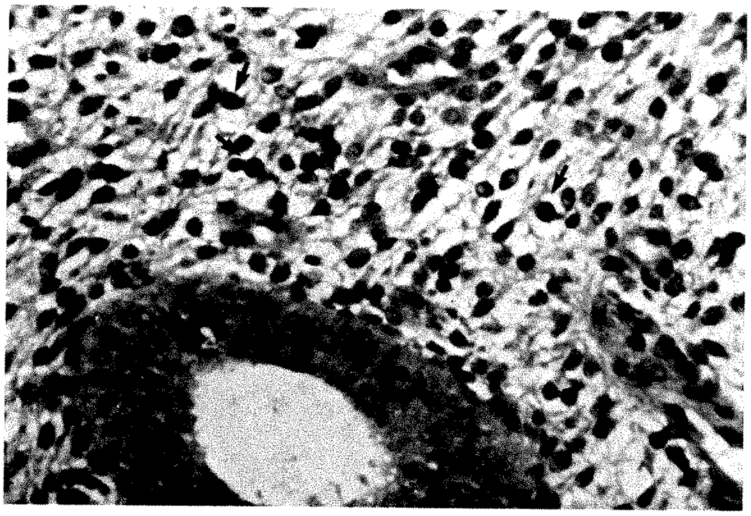

Fig. 1. Mild endometritis. Scattered plasma cells *(arrow)* in the stroma of proliferative endometrium. (Original magnification, × 132.)

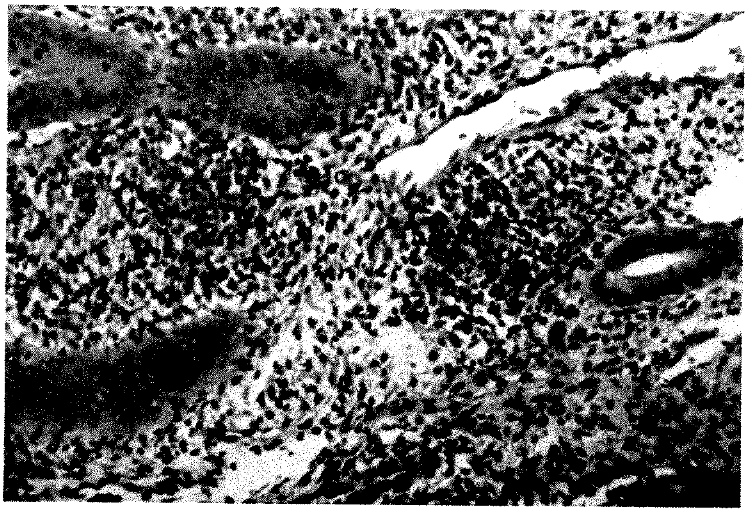

Fig. 2. Moderate endometritis. Increased stromal density with focuslike aggregates of plasma cells and lymphocytes. (Original magnification, × 66.)

to the method of Herovici[8] and also with methyl green–pyronine[9] as a special stain for plasma cells. During laparoscopy peritoneal fluid was aspirated via the laparoscope. In eight cases no fluid was seen in the cul-de-sac and 5 ml of physiologic saline solution was then injected through the laparoscope into the cul-de-sac. The fluid present in the cul-de-sac was aspirated for cytologic examination. The specimens were immediately fixed in 50% ethyl alcohol. Fluids with abundant cellular material were centrifuged and smeared; others were prepared with the Millipore technique. All slides were stained with the Papanicolaou method.[10]

Definition of endometritis. The biopsy specimens were examined by a pathologist (R. A.) who was not aware of the clinical findings. The histopathologist's diagnosis of endometritis was based on the identification of plasma cells. Endometritis was classified as mild, moderate, or severe. In mild endometritis the inflammatory infiltrate was slight and scattered in the superficial part of usually edematous endometrial stroma. Only focal aggregates formed by a few plasma cells were seen (Fig. 1). In all cases the presence of plasma cells was confirmed with methyl green–pyronine staining. The biopsy evidence of moderate endometritis was based on assessment of quantitively more marked infiltration of plasma cells that were seen evenly throughout the biopsy specimen (Fig. 2). Lymphocytes and plasma cells were also found in larger focuslike aggregates which in some places became confluent. The increase in density of the endometrial stroma was more

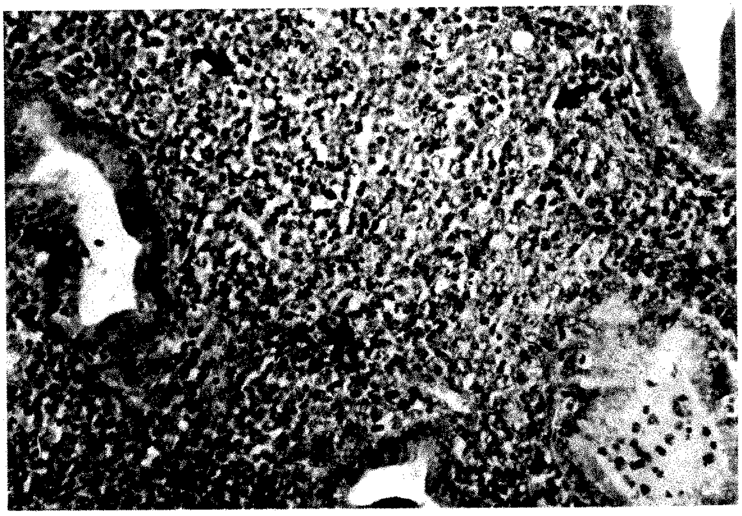

Fig. 3. Severe endometritis. Follicular collection *(arrows)* of transformed lymphocytes surrounded by dense infiltrate of plasma cells. (Original magnification, ×66.)

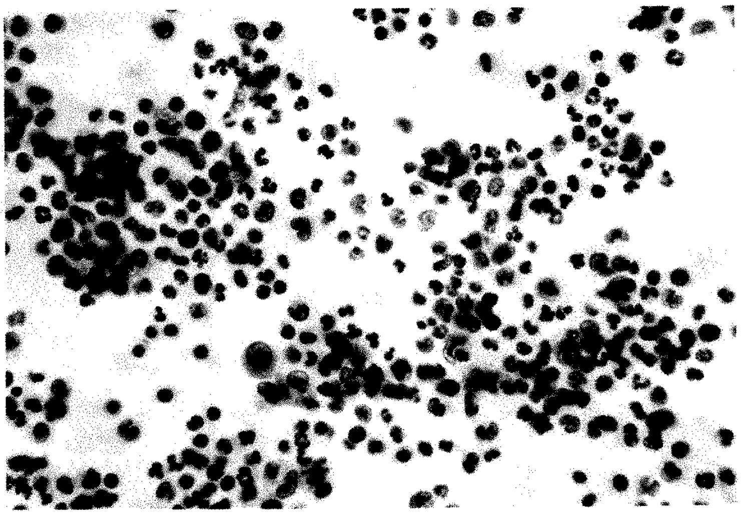

Fig. 4. Inflamed peritoneal fluid. An admixture of mesothelial cells, polymorphonuclear leukocytes, histiocytes, and monocytoid cells. (Original magnification, ×132.)

prominent than in the mild form. Endometritis was considered severe when pronounced diffuse infiltration of plasma cells was seen with replacement of endometrial stroma over all of the biopsy specimen. Rounded follicular accumulations formed by large transformed lymphocytes were observed, often with several plasma cells surrounding these structures (Fig. 3). In addition to plasma cells other inflammatory cells such as lymphocytes and neutrophils were more abundant. In severe endometritis the associated inflammatory parameters such as focal eosinophilia of the superficial epithelium and focal infiltration of endometrial glands by neutrophils[11] were more prominent than in the moderate grade of endometritis. Endometrial granulomas were not seen in any of the cases.

Definition of cytologic manifestation of peritoneal fluid inflammation. The peritoneal fluid was considered inflamed when the cellularity was increased, especially the proportion of neutrophils, eosinophils with monocytoid cells, and desquamated mesothelial cells (Fig. 4). In some cases a slight mesothelial atypia was also seen. A small number of various cell types in the specimen was considered normal.

Laparoscopic technique and definition of acute salpingitis. Laparoscopy was performed with the patient under general anesthesia with controlled respiration. Pneumoperitoneum was established by a Verres needle and insufflation of 3 to 4 L of carbon dioxide. A trocar was then introduced through the abdominal wall just below the umbilicus, and the laparoscope with oper-

Table I. Laparoscopic findings among eight women without acute salpingitis

Finding	*No.*
Ovarian cyst	4
Unruptured	1
Ruptured	3
Pelvic adhesions	1
Ectopic pregnancy	1
Normal	2

ating unit (Wolf Co., Knittlingen, West Germany) was inserted. A second puncture in the lower quadrant was made for inserting an ancillary instrument for manipulating the tubes or ovaries to improve visualization. The grade of salpingitis was assessed according to the laparoscopic criteria of mild, moderate, or severe salpingitis as previously described.[7] After the pelvic organs had been inspected the appearance and amount of peritoneal fluid in the posterior cul-de-sac were observed and the cytologic specimen was aspirated with a laparoscopic cannula equipped with a 10 ml syringe. The appendix and the upper abdominal cavity were also visualized if possible. Laparoscopy was successful in all cases, and no complications occurred.

Results

Prevalence of acute pelvic inflammatory disease. Overall, 18 (67%) of the 27 women had acute salpingitis at laparoscopy. Salpingitis was graded as mild in nine cases, moderate in four cases, and severe in five cases. Laparoscopic findings among women who did not have salpingitis are given in Table I.

Histopathologic evidence of plasma cell endometritis was found in 19 (70%) cases. Endometritis was graded as mild in six cases, moderate in four cases, and severe in nine cases.

Cytologic examination of peritoneal fluid showed an inflammatory cellular pattern in 10 (67%) of the 15 cases in which adequate cytologic specimens were obtained.

Correlation of endometrial inflammation with tubal inflammation. Endometrial biopsy specimens revealed plasma cell endometritis in 16 (89%) of the 18 cases with acute salpingitis at laparoscopy (Table II). All women who had severe salpingitis at laparoscopy had endometritis as well. However, plasma cell endometritis was also found in three (33%) of the nine women who did not have salpingitis at laparoscopy. Of the latter three women, one had mild, one had moderate, and one had severe endometritis on endometrial biopsy. Their contraceptive methods were oral contraceptives, the barrier (condom) method, and the intrauterine contraceptive device, respectively.

On the other hand, two (25%) of the eight women without endometritis nonetheless had salpingitis at laparoscopy (one had mild and one had moderate salpingitis).

Thus, in comparison to laparoscopically detected salpingitis, endometritis detected at biopsy had a sensitivity of 89%, a specificity of 67%, a positive predictive value of 84%, and a false negative rate of 22% in the diagnosis of acute pelvic inflammatory disease.

Correlation of inflammatory peritoneal fluid cytologic testing with tubal inflammation. Nine (75%) of the 12 women with acute salpingitis at laparoscopy had inflammation detected by cytologic testing of the peritoneal fluid (Table III). All women with severe salpingitis at laparoscopy had such inflammation. Inflammation was also seen in one of the three women who did not have salpingitis at laparoscopy. She also did not have endometritis, and a ruptured right ovarian cyst was detected at laparoscopy. On the other hand, three (60%) of the five women who did not have inflammation on cytologic testing of the peritoneal fluid aspirate nonetheless had acute salpingitis at laparoscopy. One of them had mild salpingitis, and two demonstrated moderate salpingitis. Two of them lacked findings of endometritis as well. Thus, in comparison to laparoscopically detected salpingitis, the presence of an inflammatory cell pattern in the peritoneal fluid aspirate had a sensitivity of 75%, a specificity of 67%, a positive predictive value of 90%, and a false negative rate of 25% in the diagnosis of acute pelvic inflammatory disease.

Comment

Laparoscopy is a prerequisite for the diagnosis of acute salpingitis. Although salpingitis is often used synonymously with pelvic inflammatory disease the former should preferably be used in visually confirmed cases only. Laparoscopic studies have shown that the diagnosis based on pelvic examination and laboratory tests alone is often erroneous in cases of suspected pelvic inflammatory disease.[2-6] If the pathogenesis of acute pelvic inflammatory disease in most cases is the intraluminal spread of the inflammation from the cervix and vagina to the upper genital tract,[12] endometrial biopsy to demonstrate endometritis might be an alternative method to identify upper genital tract infection among women with suspected acute salpingitis. Many patients with lower genital tract infection who have findings on history and pelvic examination that are suggestive of acute pelvic inflammatory disease nonetheless have normal laparoscopic findings.[3, 4] Although such cases have been termed as lower genital tract infection, they are often associated with clinical findings suggesting early upper genital tract infection, such as elevation of the erythrocyte sedimentation rate or peripheral blood leukocyte count. Many such patients may actually have

Table II. Correlation of laparoscopy with endometrial biopsy

Laparoscopic finding	*Biopsy finding*				*Total*
	Endometritis			*No endometritis*	
	Severe	*Moderate*	*Mild*		
Severe salpingitis	4	1	0	0	5
Moderate salpingitis	2	0	1	1	4
Mild salpingitis	2	2	4	1	9
No salpingitis/other	1	1	1	6	9
Total	9	4	6	8	27

endometritis rather than lower genital tract infection only. In fact, one recent study demonstrated evidence of endometritis on endometrial biopsy in 40% of 35 patients referred for suspected cervicitis.[13]

To address this question further we designed a prospective study with concomitant laparoscopy and endometrial biopsy performed among women with suspected acute pelvic inflammatory disease. We found that 18 of the 27 women initially thought to have acute pelvic inflammatory disease had acute salpingitis at laparoscopy; thus, the diagnosis made by clinical bimanual examination had a positive predictive value of only 67%. This is in accordance with previous studies.[3,4] We also found that 16 of these 18 women had plasma cell endometritis, which gives sensitivity of 89% for endometrial biopsy in detecting acute salpingitis. This study demonstrates that nonpuerperal endometritis is an entity associated with acute salpingitis, probably representing an intermediate stage between cervicitis and salpingitis. There may be multiple reasons why endometritis was not demonstrated in all patients with acute salpingitis at laparoscopy. Endometritis may be a patchy lesion and a single-strip biopsy that contains only a small portion of the endometrium may not contain a sufficient quantity either to establish or to disprove a diagnosis of endometritis. Alternatively, acute salpingitis may develop in some cases primarily by the parametrial route of ascending inflammation.[2,14] This would explain why acute exosalpingitis is seen in the absence of plasma cell endometritis, the latter of which would indicate intraluminal spread of the lower genital tract infection. A third possibility is a false positive finding of mild acute salpingitis at laparoscopy although no data exist so far to support this hypothesis. Perhaps signs of a past episode of salpingitis such as increased vascularity and erythema might stimulate mild acute salpingitis. Furthermore, edema and erythema of the fallopian tubes may vary according to the phase of the menstrual cycle.

We found three cases (33%) of endometritis among the nine women who did not have salpingitis. One of these three women had an intrauterine device inserted. Overall, there were six (32%) intrauterine device users among the 19 women who had plasma cell endometritis versus two (25%) users among the eight women who did not have endometritis. Thus intrauterine device use was not associated with plasma cell endometritis alone in our study population. Our finding supports the hypothesis of the presence of early pelvic inflammatory disease, that is, endometritis, among some women who have normal tubes at laparoscopy but who nonetheless have clinical signs suggestive of pelvic inflammatory disease. Further study is now in progress to test this finding in a larger population. This finding also supports earlier results of a high rate of endometritis among women with cervicitis in the absence of clinical signs of acute pelvic inflammatory disease.[13] Thus good diagnostic tests are necessary to identify women with early pelvic inflammatory disease who are at high risk to develop endosalpingitis often followed by long-term sequelae. One such test clearly is proper identification and management of mucopurulent cervicitis[15] and a second may be more liberal use of endometrial biopsy in such cases. The importance of endometrial biopsy is already recognized for the evaluation of patients with infertility and abnormal uterine bleeding, and it can be performed in the office without anesthesia. Although not proved it is conceivable that early endometritis can cause infertility since approximately half of women with tubal infertility do not have a history of frank pelvic inflammatory disease.[16-18]

Table III. Correlation of inflammatory peritoneal fluid cytologic findings with tubal inflammation

Acute salpingitis	*Inflammatory cytologic findings*		*Total*
	Present	*Absent*	
Present	9	3	12
Absent	1	2	3
Total	10	5	15

We also studied the cellular pattern of peritoneal fluid to find out if the presence of an inflammatory cellular pattern confirms tubal infection or if it might help to identify those cases of salpingitis that are false positive at laparoscopy. First, we found that 75% of women with salpingitis, including all those who had severe salpingitis, had cytologic manifestation of inflammation in the peritoneal fluid. Second, 60% (three of five) of women who did not have cytologic inflammation had salpingitis at laparoscopy. Two of them also did not have endometritis. This might again indicate false positive laparoscopic findings, although no study has yet addressed this question.

This study demonstrates that nonpuerperal endometritis is an entity associated with pelvic inflammatory disease and most likely represents an intermediate stage between cervicitis and salpingitis. Endometrial biopsy seems to be a novel approach as an alternative to laparoscopy in the diagnosis of acute pelvic inflammatory disease. Reasons for reluctance to perform a biopsy of the endometrium in the presence of acute infection may well be exaggerated. In this and another recent related study,[13] the patients did not receive antibiotics prior to the laparoscopy and endometrial biopsy. Therapy was started immediately when these invasive procedures were performed, and no complications possibly related to these procedures were identified. Furthermore, endometrial biopsy (or curettage) is frequently used to evaluate patients with other gynecologic disturbances such as infertility and irregular uterine bleeding often associated with acute or chronic endometrial infection. Endometrial biopsy is a relatively simple procedure, and there will be only a 24- to 48-hour delay of histopathologic diagnosis.

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Physiologic concentrations of dopamine fail to suppress prolactin secretion in patients with idiopathic hyperprolactinemia or prolactinomas

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Several investigators have suggested that normal responsiveness to dopamine is exhibited by pituitary lactotrophs in patients with idiopathic hyperprolactinemia and prolactinomas. These studies, however, have employed dopamine infusion rates that produced supraphysiologic serum dopamine concentrations. In order to further examine this issue, we infused graded doses of dopamine to normal men and women as well as to patients with idiopathic hyperprolactinemia and prolactinomas. Dopamine infusion rates as low as 0.004 μg/kg/min, which were associated with physiologic serum dopamine levels, produced significant ($p < 0.01$) suppression of prolactin in normal women and in normal males ($p < 0.05$). In contrast, a 10-fold increase in the dopamine infusion rate, 0.04 μg/kg/min, was required in the hyperprolactinemic subjects to produce prolactin suppression similar to that found in the control subjects. Hence, prolactin secretion in both tumors and other hyperprolactinemic states is associated with a resistance to suppression by dopamine. (AM J OBSTET GYNECOL 1985;151:651-5.)

Key words: Prolactin, dopamine, prolactinomas, idiopathic hyperprolactinemia

Dopamine exerts a critical role in the regulation of prolactin secretion by acting as a prolactin inhibitory factor.[1, 2] In an attempt to help clarify the mechanism responsible for excessive prolactin secretion in various hyperprolactinemic states, dopamine infusions have been utilized.[3-9] Intravenous dopamine administration has been reported to exert a normal pituitary inhibitory effect on the secretion of prolactin in patients with idiopathic hyperprolactinemia and prolactinomas.[3-6] Other studies have suggested, however, that women with idiopathic hyperprolactinemia have a lactotrophic resistance to dopamine.[8, 9] Most of the earlier investigations have employed continuous infusions of dopamine ≥4 μg/kg/min.[3-14] These infusion rates produce pharmacologic serum dopamine concentrations.[7] Only recently have studies utilized lower dopamine infusion rates, which, however, still produce supraphysiologic serum dopamine concentrations.[7-9] In this study we have employed dopamine infusion rates of 0.4 to 0.0004 μg/kg/min to determine a dopamine infusion rate that would produce physiologic dopamine concentrations as well as prolactin inhibition in normal subjects. This information was then used to assess the sensitivity of pituitary lactotrophs to dopamine infusion rates that produce physiologic serum dopamine concentrations in patients with idiopathic hyperprolactinemia and prolactinomas.

Material and methods

Subjects. Fourteen healthy women and five healthy men within 10% of ideal body weight were studied (Table I). The subjects were on an ad lib diet prior to the study, and no subject was taking any medication. All were euthyroid and had no evidence of renal or hepatic dysfunction. In addition, the women all had regular menstrual cycles, and none had a family history of breast carcinoma.

Fifteen hyperprolactinemic women were also studied. Eight of the hyperprolactinemic women had prolactinomas (five surgically documented, one patient with a serum prolactin level of 473.0 and an enlarged sella who refused therapy, and two who had received radiotherapy). Seven women who were evaluated had idiopathic hyperprolactinemia (see Table I). Two male patients with surgically documented prolactinomas were also studied. None of the patients had evidence of hypothyroidism or hypoadrenalism at the time of the study.

Testing procedure. All patients were admitted to the Ohio State University Hospitals Clinical Research Center after an overnight fast, and informed consent was obtained. A heparin-lock cannula was inserted into a

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Table I. Serum prolactin levels in control and hyperprolactinemic subjects

	Normal men	*Normal women*	*Patients with prolactinomas*	*Idiopathic hyperprolactinemia patients*
Number	5	14	10	7
Mean age (yr)	24.6	24.4	32.5	26.4
Range	22-28	19-46	17-51	23-38
Mean prolactin level (ng/ml)	11.6	10.7	167.9	86.8
Range	6.8-17.6	6.7-20	30.3-473.7	29.5-207.9

forearm vein between 9 and 9:30 AM. On separate days, dopamine hydrochloride (Intropin), diluted in 150 ml of normal saline solution was infused from 1000 to 1300 hours at constant rates of 0.4 μg, 0.04 μg, 0.004 μg, or 0.0004 μg/kg/min through a butterfly needle. The dopamine infusions were started at 10 AM because the nadir of serum prolactin levels in humans usually occurs at this time of day, and hence further decreases in the serum prolactin levels should be due to the infusion and not secondary to normal diurnal variation.[15] Four normal subjects who received intravenous normal saline solution did not exhibit a significant fall in serum prolactin levels over 180 minutes (data not shown). In all subjects the blood pressure and heart rate were recorded at 30-minute intervals during the course of the experiments. Dopamine infused at the indicated rates induced no perceivable changes in cardiovascular function. The subjects continued to fast throughout the experiment, and blood samples were obtained for prolactin level at 0, 30, 60, 90, 120, 150, and 180 minutes and for dopamine level at 0, 60, 120, and 180 minutes. The serum was frozen immediately after it was obtained.

Assays and statistical methods. Prolactin was assayed by a homologous radioimmunoassay as previously described.[15] The intraassay and interassay coefficients of variation for this assay were <10%. Dopamine was measured by a radioenzymatic assay (Cat-A-Kit, Upjohn Co.). The intraassay and interassay coefficients of variation for this assay in our laboratory were 5% and 13%, respectively. Serum prolactin levels are expressed in terms of percent change from baseline because of the marked differences in basal prolactin levels in the hyperprolactinemic subjects. The prolactin data were analyzed by Dunnett's multiple comparison test. A p value of <0.05 was considered significant.

Results

Normal women. Dopamine infusion rates of 0.4 μg ($n = 3$ subjects) and 0.04 μg/kg/min ($n = 10$) caused significant suppression of prolactin at 60, 90, 120, 150, and 180 minutes (Fig. 1). The dopamine infusion rate of 0.004 μg/kg/min ($n = 7$) produced suppression at 90, 120, 150, ($p < 0.01$) and 180 minutes ($p < 0.05$) whereas, the 0.0004 μg/kg/min rate ($n = 3$) produced prolactin suppression ($p < 0.01$) at 120, 150, and 180 minutes.

Dopamine levels. In six normal subjects and in three hyperprolactinemia subjects (two idiopathic, one with pituitary tumor), serum dopamine levels increased to a maximum of 52 ± 17 pg/ml (mean + SEM) with a range of 28 to 120 pg/ml in the normal subjects and 86 ± 36 pg/ml with a range of 40 to 162 pg/ml in the hyperprolactinemic subjects at an infusion rate of 0.004 μg/kg/min (Fig. 2). These dopamine levels were not statistically different.

Idiopathic hyperprolactinemic women. In the six subjects with idiopathic hyperprolactinemia, the dopamine infusion rate of 0.004 μg/kg/min, which produced significant suppression of prolactin in normal females, did not cause significant inhibition of prolactin at any interval during the infusion (Fig. 3). Several subjects within the group, however, did exhibit normal prolactin suppression. Dopamine infusion rates of 0.04 μg/kg/min, however, produced suppression of prolactin ($p < 0.01$) at all time points between 30 and 180 minutes in this group of patients.

Female prolactinoma patients. In a similar manner to the idiopathic hyperprolactinemic women, the prolactinoma patients ($n = 4$) did not exhibit significant prolactin suppression at any interval during the 0.004 μg/kg/min rate (Fig. 3). One of the four prolactinoma patients, however, exhibited normal prolactin suppression to the 0.004 μg/kg/min dopamine infusion. Both the 0.4 μg/kg/min and the 0.04 μg/kg/min rates ($n = 3$, 7 respectively) produced significant prolactin suppression at 30 minutes ($p < 0.01$).

Normal men. Dopamine infusion rates of 0.4 μg/kg/min in 3 male subjects inhibited prolactin levels ($p < 0.01$) at all time points between 20 and 180 minutes. The 0.04 μg/kg/min rate ($n = 5$) caused prolactin suppression ($p < 0.01$) at all time points between 60 and 180 minutes, whereas the 0.004 μg/kg/min rate produced significant ($p < 0.05$) prolactin suppression at only the 120 and 150 minute time points (Fig. 4).

Male prolactinoma patients. Dopamine infused at a

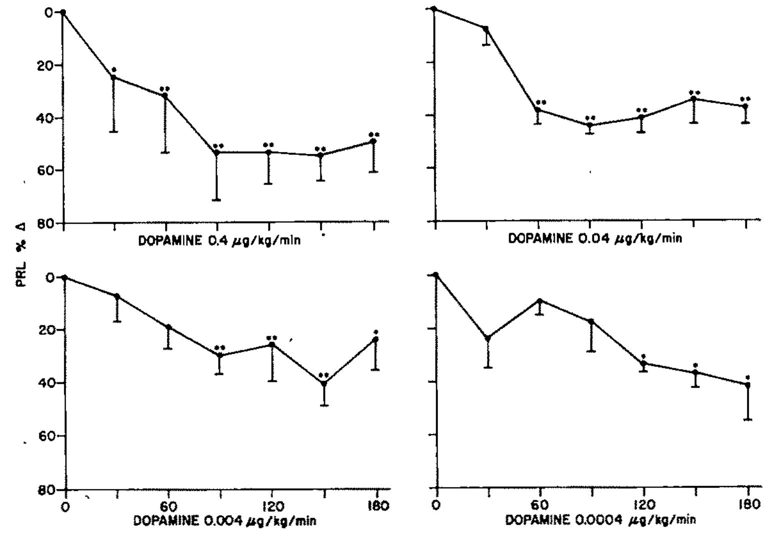

Fig. 1. The mean ± SEM percent decrease in serum prolactin *(PRL)* levels after dopamine was infused at 0.4 μg (n = 3), 0.04 μg (n = 10), 0.004 μg (n = 7) and 0.0004 μg/kg/min (n = 3) for 3 hours in normal women. *, $p < 0.05$; **, $p < 0.01$.

rate of 0.004 μg/kg/min caused a 47% decrease of prolactin in one subject and only a 21% decrease in the other male patient.

Comment

In this study we have demonstrated that a dopamine infusion rate of 0.004 μg/kg/min produces serum concentrations of dopamine found in normal subjects during standing and exercise.[16] These physiologic concentrations of dopamine were able to significantly inhibit prolactin secretion in normal men and women (Figs. 1 and 4). Previous investigations have used dopamine infusion rates that have achieved supraphysiologic concentrations of serum dopamine during the infusion. Those investigations utilizing dopamine infusion rates of 4 μg/kg/min were able to significantly suppress prolactin secretion in normal subjects as well as those with hyperprolactinemia.[3-6] Other studies employing dopamine infusion rates of 0.02 to 0.004 μg/kg/min have suggested that patients with idiopathic hyperprolactinemia have diminished prolactin inhibition to dopamine.[8, 9] One of these studies also suggested that patients with prolactinomas have normal responsiveness to dopamine.[8]

In this study we found a lack of suppression of serum prolactin levels to physiologic levels of dopamine in patients with idiopathic hyperprolactinemia and prolactinomas. Our findings of dopamine resistance to prolactin suppression in our hyperprolactinemic population differ from other reports that employed higher dopamine infusion rates.[3-6] Our findings in patients with idiopathic hyperprolactinemia are similar to those studies,[8, 9] which with use of lower dopamine infusion rates have found a resistance of prolactin suppression to dopamine. The discrepancy between our results and those reported by Serri et al.[8] in patients with macroprolactinomas might be explained by the fact that their lowest dopamine infusion rate (0.02 μg/kg/min)

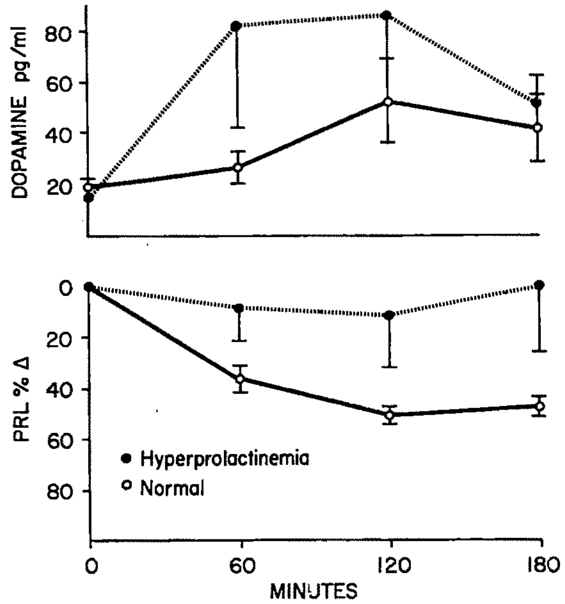

Fig. 2. Physiologic plasma concentrations of dopamine were produced in normal (n = 6) and hyperprolactinemic (n = 3) subjects with a 0.004 μg/kg/min dopamine infusion rate over 180 min. Mean ± SEM serum prolactin *(PRL)* levels decreased significantly ($p < 0.01$) at 60, 120, and 180 min in the normal subjects but not in the hyperprolactinemic subjects.

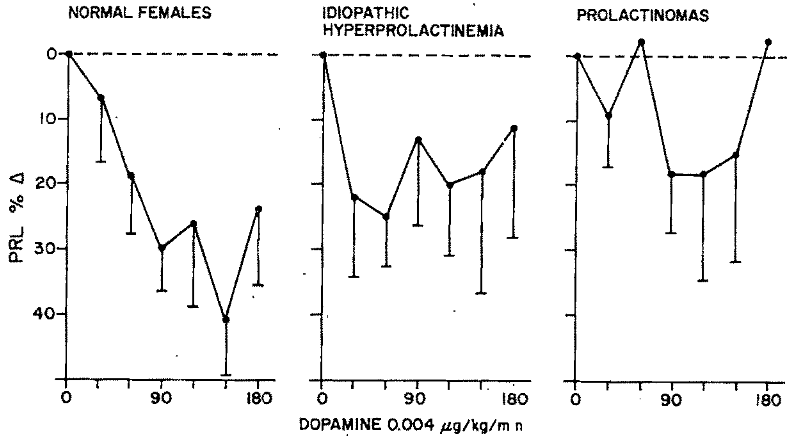

Fig. 3. A 0.004 μg/kg/min dopamine infusion rate decreased prolactin *(PRL)* secretion in normal women (n = 7) but failed to produce a significant prolactin decrease in women with idiopathic hyperprolactinemia (n = 6) or prolactinomas (n = 4). *, $p < 0.05$; **, $p < 0.01$.

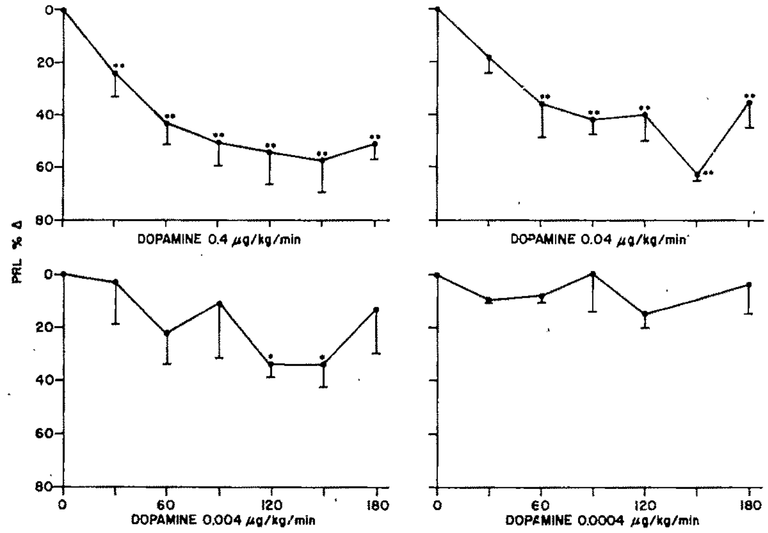

Fig. 4. The mean ± SEM percent decrease in serum prolactin *(PRL)* levels after dopamine infusion at 0.4 μg (n = 3), 0.04 μg (n = 5), 0.004 μg (n = 4) and 0.0004 μg/kg/min (n = 2) for 3 hours in normal men. *, $p < 0.05$; **, $p < 0.01$.

achieved pharmacologic serum dopamine concentrations. The dopamine levels found in their study were 10-fold higher than the dopamine concentrations noted in our subjects who received a dopamine infusion rate of 0.004 μg/kg/min.

There are several possible explanations for the failure of low-dose dopamine infusions to inhibit prolactin secretion in hyperprolactinemic states. First, the resistance could be related to increased clearance of dopamine in the hyperprolactinemic subjects. Our data and a previous report,[7] however, have found either similar or elevated serum dopamine levels in hyperprolactinemic subjects following a dopamine infusion. Second, decreased prolactin clearance in the hyperprolactinemic subjects could explain these differences. However, prolactin clearance has been shown to be normal in hyperprolactinemic subjects.[7, 12] Third, the prolactin-resistant state could be a consequence of altered central nervous system function produced by the hyperprolactinemia. A recent study, however, using L-dopa in females with postpartum hyperprolactinemia, documented that these women had normal central dopaminergic inhibition of prolactin release.[17] Finally, the prolactin-resistant state could be related to a dopamine receptor and/or postreceptor alteration in the pituitary lactotroph population. Supporting this hypothesis have been observations reported from our laboratory as well as others documenting in vitro resistance of prolactin secretion to dopamine and its agonists in rodent[18-20] and human[21, 22] pituitary cultures. Also, we have published findings from one subject[22] and recently evaluated another in whom resistance of prolactin secretion to dopamine infusion was noted in the patients prior to pituitary tumor excision. Subsequent evaluation of the pituitary tumor in culture demonstrated similar resistance of prolactin secretion to inhibition by dopamine.

In this study we also observed a few individuals with idiopathic hyperprolactinemia and prolactinomas who demonstrated a normal prolactin response at several time points to the 0.004 μg/kg/min dopamine infusion. This finding suggests that the hyperprolactinemic population is heterogenous in regard to dopamine-prolactin interaction, which is similar to the conclusion previously made by Thorner et al.[23] who used bromocriptine to suppress prolactin secretion in hyperprolactinemic subjects. In addition, the blunted prolactin response to dopamine suggests that pituitary and/or other central nervous system defects may be present in the hyperprolactinemic patient population. Further investigation will be required, however, to clarify this hypothesis.

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Statistical comparison of Pearl rates

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Statistical procedures for hypothesis testing and interval estimation of the difference in a pair of Pearl rates are presented. The p value of the test statistic is evaluated exactly from the binomial distribution or approximately from the standard normal distribution. Interval estimation is prcvided by the test-based method of Miettinen. The procedures are applied to two published data sets, and the conceptual link between the Pearl rate and life-table methods is discussed. (AM J OBSTET GYNECOL 1935;151:656-9.)

Key words: Pearl rate, hypothesis test, confidence interval, life table

The Pearl rate is frequently used in clinical studies to summarize contraceptive efficacy. It estimates the number of pregnancies in 100 woman years of method use and is calculated as follows:

$$\frac{\text{Number of pregnancies}}{\text{Number of woman months of pregnancy risk}} \times 1200$$

The denominator is the sum of the number of months each woman used the contraceptive method up to and including the month of conception or until termination of use.

Before the widespread introduction of life-table techniques in the early 1960s, the Pearl rate was the standard measure of contraceptive effectiveness. The Pearl rate is a good estimator of the risk of pregnancy if it can be assumed that all the women under observation have the same probability of pregnancy and of loss to follow-up and that these probabilities remain constant over the observation period. The latter assumption is often invalid, which causes the Pearl rate to be biased.[1,2] Despite this methodologic flaw, the Pearl rate has been a survivor[3]: recent publications include it in addition to life-table probabilities.[4,5] In part, the Pearl rate is appealing because of its relative simplicity both computationally and interpretatively. Additionally, since it was the standard measure for three decades, continued use of the Pearl rate provides a connection to earlier studies for which life-table probabilities cannot be calculated in retrospect.

In comparative clinical trials it is routine to test for differences in life-table probabilities, and statistical tests are well defined. In studies in which Pearl rates have been compared, it was under the assumption that they were binomial proportions, and a statistical test of the independence of contraceptive method and pregnancy was applied. However, Pearl rates are not binomial proportions.[1,6]

As an alternative to the binomial model, Potter and Sagi[1] recommended treating the Pearl rate as a ratio estimator with variance calculated by sample survey methods. This approach does not require identification of the type of distribution governing the number of pregnancies and takes into account the statistical dependence between the number of pregnancies and the number of follow-up months. The disadvantage with the approach is that in order to estimate the variance it is necessary to use essentially the same information required by life-table estimates.

In 1976 Miettinen[7] attached the label "incidence density" to measures like the Pearl rate and offered a procedure for interval estimation. Recently a method was presented to test the difference in two incidence densities.[6] In the sections that follow, these new methods will be described and illustrated with the use of data from two published reports.

Method

The data needed to calculate the Pearl rates in a comparative study are shown in Table I. The number of pregnancies in the study period starting at t_0 and ending at t is n, which is the sum of n_1 pregnancies in L_1 woman months of follow-up for contraceptive C_1, and n_2 pregnancies in L_2 follow-up months for contraceptive C_2. L represents the total woman months of follow-up ($L = L_1 + L_2$). The respective Pearl rate estimates for C_1 and C_2 are as follows:

$$r_1 = \frac{n_1}{L_1} \times 1200 \text{ and } r_2 = \frac{n_2}{L_2} \times 1200$$

In epidemiologic terms the ratios $d_1 = n_1/L_1$ and $d_2 = n_2/L_2$ are the respective estimates of incidence density of pregnancy for C_1 and C_2 with the result that $r_1 = d_1 \times 1200$ and $r_2 = d_2 \times 1200$.

Hypothesis test. The null hypothesis is $H_0: R_1 = R_2$

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Table I. Data summary for calculating Pearl rates for a comparative study

	Contraceptive method		
	C_1	C_2	*Total*
Number of pregnancies in study period (t_0-t)	n_1	n_2	$n = n_1 + n_2$
Woman months of risk in study period (t_0-t)	L_1	L_2	$L = L_1 + L_2$
Pearl rate	$\frac{n_1}{L_1} \times 1200$	$\frac{n_2}{L_2} \times 1200$	—

C_i = Contraceptive i; n_i = the number of pregnancies occurring in contraceptive group i; L_i = the number of woman months of risk accumulated for contraceptive group i; t_0 = the beginning of the study period; t = the end of the study period.

or equivalently $H_0: D_1 = D_2$ where R_1 and R_2 represent the true Pearl rates and D_1 and D_2 are the true incidence densities of pregnancy for the two contraceptives. The null hypothesis of equal Pearl rates is tested by assuming that the n observed pregnancies can be attributed to one of the two methods according to an independent Bernoulli process.[6] If the null hypothesis is true, the probability that a given pregnancy belongs to a user of contraceptive C_1 is estimated by $p = L_1/L$ and the probability of not belonging to C_1 and therefore belonging to C_2 is estimated by $q = L_2/L$. Thus equal Pearl rates imply that the proportion of the total of pregnancies attributable to method C_1 equals the proportion of total risk time attributable to that method ($n_1/n = L_1/L$).

The p value for the test will be established for the one-sided alternative hypothesis that the Pearl rate for C_1 is larger than that for C_2. The p value can be calculated exactly by evaluating the binomial sum

$$\sum_{i=n_1}^{n} \binom{n}{i} p^i q^{n-i}$$

where

$$\binom{n}{i} = n!/i!(n - i)!$$

Tables of the binomial sum for values of n and p are readily available.[8] If np and nq both exceed 5, an approximate p value can be determined by use of the test statistic $z = (n_1 - np)/\sqrt{npq}$, which has approximately a standard normal distribution under the null hypothesis. The p value is evaluated in the usual way by use of a standard normal distribution table.[9]

Confidence interval. Two-sided confidence intervals for the difference $R_1 - R_2$ and ratio R_1/R_2 of two Pearl rates are constructed by the test-based procedure.[7] The method is appropriate for use with large samples, since the estimate of standard deviation is a function of the z statistic presented earlier for the large sample hypothesis test. The test-based confidence interval for the difference in two Pearl rates is $(r_1 - r_2)(1 \pm Z_{1-\alpha/2}/|z|)$ where $Z_{1-\alpha/2}$ is the percentile of the standard normal distribution that is used to give a $(1 - \alpha) \times 100\%$ confidence interval and $|z|$ is the absolute value of the z statistic described in the section on hypothesis tests. The test-based confidence interval for the ratio of two Pearl rates is $(r_1/r_2)^{(1 \pm Z_{1-\alpha/2}/|z|)}$.

Examples

In a report from the Oxford Family Planning Association Contraceptive Study covering the period from May, 1968, to April, 1975,[10] the Pearl rate was used exclusively as the measure of contraceptive use-effectiveness. Confidence intervals for individual method rates were calculated by assuming that the Pearl rates were binomial proportions. Differences in Pearl rates were not compared statistically. Tables IIA and IIB show data on contraceptive use-effectiveness taken from Table 28 of the interim report rearranged in a convenient computational form.

To test whether the Pearl rate associated with Lippes Loop A or B exceeds that of Lippes Loop C or D, we first calculated p and q from the woman months of observation as $p = 4992/67{,}980 = 0.073$ and $q = 1 - p = 0.927$ (Table IIA). Since $np = 118 \times 0.073 > 5$ and $nq = 118 \times 0.927 > 5$, the test statistic was calculated in three decimal–place precision as follows:

$$z = [17 - (118 \times 0.073)]/\sqrt{118 \times 0.073 \times 0.927} = 2.968$$

From a table of the standard normal distribution,[9] the p value was determined to be 0.002. The 95% confidence interval for the ratio of Pearl rates was $(4.086/1.924)^{(1 \pm 1.96/2.968)}$, or 1.3-3.5.

Because of the few pregnancies among women in the two groups of oral contraceptive users (Table IIB), the exact method of computing the p value was used to test whether the Pearl rate for progestogen-only users is greater than that for users of combination pills. Here $n_1 = 6$ and $n = 9$, so $p = 2436/12{,}708 = 0.192$ and $q = 1 - p = 0.808$. The p value was given by

$$\sum_{i=6}^{9} \binom{9}{i} 0.192^i \, 0.808^{9-i}$$

which was evaluated approximately as 0.002 by rounding the values of p to 0.19 and q to 0.81 and by referring to a table of binomial probabilities.[8]

A second example comes from a study of intrauterine

Table IIA. Use-effectiveness of intrauterine contraceptive devices investigated in the Oxford Family Planning Association Study*

	Lippes Loop		
	A or B	*C or D*	*Total*
Number of pregnancies	17	101	118
Woman months of observation	4992	62,988	67,980
Pearl rate	4.09	1.92	—

*Source: Table 28 of Reference 10.

Table IIB. Use-effectiveness of oral contraceptives investigated in the Oxford Family Planning Association Study*

	Progestogen only	*Combined >50/μg estrogen*	*Total*
Number of pregnancies	6	3	9
Woman months of observation	2436	10,272	12,708
Pearl rate	2.96	0.35	—

*Source: Table 28 of Reference 10.

Table III. Clinical trials data on the use-effectiveness of two intrauterine contraceptive devices*

	SCS-D	*TCu-200*	*Total*
Number of pregnancies	11	10	21
Woman months of observation	3492	4589	8081
Pearl rate	3.78	2.61	—

*Source: Table 2 of Reference 11.

contraceptive device efficacy which was analyzed by life-table methods (Table III).[11] To test whether the Pearl rate for the SCS-D intrauterine contraceptive device is larger than that for the TCu-200 intrauterine contraceptive device, we calculated that p = 3492/8081 = 0.432 and q = 1 − p = 0.568. Since n = 21, both np and nq exceeded 5, and the z statistic calculated in three decimal–place precision was

$$z = [11 - (21 \times 0.432)] \Big/ \sqrt{21 \times 0.432 \times 0.568} = 0.849$$

with a p value of 0.198. The 95% confidence interval for the ratio of Pearl rates was $(3.780/2.615)^{(1 \pm 1.96/0.849)}$, or 0.6-3.4.

Comment

In actuarial science there are links between the Pearl rate and the (cumulative) life-table pregnancy "rate"[12]; both are determined by a time-dependent function we will call the "force of pregnancy." The force of pregnancy generally varies with time and, for a specific point in time, gives the "instantaneous" rate of pregnancy. Figuratively, the force of pregnancy acts on the study subjects over a fixed period to cause pregnancy in some proportion of the women. The life-table pregnancy rate is not a rate but the probability or proportion of pregnancies in a fixed time period among a group of women who were not pregnant at the beginning of the period. It is often stated as the number of pregnancies per 100 women. By contrast, the Pearl rate measures the average force of pregnancy per fixed time period, since it is calculated as the change in the number of pregnancies per unit change in exposure months. It is expressed as pregnancies per 100 woman years of exposure.

As an example, assume that 100 women not pregnant at the beginning of an efficacy study are followed for a year and that 20 become pregnant. If no women are lost from the study for reasons other than pregnancy, the 1-year life-table pregnancy rate is 20/100 = 0.20, or 20 pregnancies per 100 woman entering the study. Further, suppose that the pregnancies were uniformly distributed over the year. This implies that the average time of pregnancy is the midpoint of the year. Thus during the period of 1 year each woman who did not get pregnant contributed 12 months of exposure, whereas each woman who became pregnant contributed, on the average, 6 months of exposure. The total woman months of exposure is (80)(12) + (20)(6) = 1080. The Pearl rate is (20/1080) × 1200 = 22, or 22 pregnancies per 100 woman years of exposure.

Assume now that the 20 pregnancies in the example occurred in the first month of the study. The 1-year life-table pregnancy rate would remain at 20 pregnancies per 100 women, but the Pearl rate would increase to (20/980) × 1200 = 24, since 20 of the women were observed for only 1 month. The 1-month life-table pregnancy rate would also be 20 per 100 women while the Pearl rate based on the first month of follow-up would balloon to (20/100) × 1200 = 240, or 240 pregnancies per 100 woman years of exposure. Even though the Pearl rate and the life-table pregnancy rate are governed by the same function, they measure different parameters and can give widely varying values.

Providing a statistical comparison of Pearl rates does not replace the need for comparing pregnancy rates calculated by life-table methods. In studies that report both measures but rely on life-table rates for a statistical comparison of efficacy, it may be useful, in a descriptive sense, to present a confidence interval for the difference or the ratio of Pearl rates. Since Pearl rates usually change with time, it is important that they be compared at the same time point. If Pearl rates are the only measures of efficacy used and a comparison is desired, the methods presented here are appropriate.

Most investigators are interested in comparing Pearl rates two at a time. However, the method can be extended to test simultaneously the equality of more than two Pearl rates. The extension involves assembling the data in a way similar to Table I but with as many columns as there are contraceptives to be compared. If the Pearl rates are equal, the proportion of the total pregnancies attributable to each contraceptive method equals the proportion of total exposure time attributable to the respective method, and the multinomial probability distribution can be used to evaluate the statistical significance of any differences. We have not illustrated the extension because the problem is not encountered very often and because it would add to the complexity of the paper. We would be pleased to provide the details of applying the extension to any interested reader.

Although the Pearl rate is specific to pregnancy, the concept of incidence density can be applied to other risks that are of interest in clinical studies of contraceptives. The formulas given earlier for comparing Pearl rates can be used to compare incidence densities for other risks.

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Complete heart block in a fetus associated with maternal Sjögren's syndrome

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This report illustrates the case of a fetus who was diagnosed by M-mode echocardiography, at the twentieth week of gestation, as having a complete heart block. The mother had a positive antinuclear antibody test result with antibodies directed against the Ro (SSA) and La (SSB) antigen system consistent with the diagnosis of Sjögren's syndrome. Management and outcome of the pregnancy are discussed.
(AM J OBSTET GYNECOL 1985;151:660-1.)

Key words: Fetal heart block, Sjögren's syndrome

In this article we report a case in which a fetus was diagnosed by M-mode echocardiography as having a complete heart block at the twentieth week of gestation. The mother had a positive antinuclear antibody test result with antibodies directed against the Ro (SSA) and La (SSB) antigen system; these results are consistent with the diagnosis of Sjögren's syndrome. The management of the case and the outcome of the pregnancy are discussed.

Case report

Maternal history. Mrs. D. B., a 30-year-old woman, gravida 4, para 3, was referred to the Oregon Health Sciences University because of a fetal cardiac arrhythmia detected by Doppler scanning during a routine antenatal check at 20 weeks of gestation. She gave no relevant past history except for an onset of herpes zoster 2 years prior to this pregnancy, which resolved. She had no risk factor for structural or functional heart disease. She had no specific symptoms of collagen vascular disorders although she gave a history of episodic swelling of the parotid glands. A physical examination was normal. A filter paper was inserted into the unanesthetized conjunctival sac at the outer third of the lower lid (Schirmer's test) and showed no tear production at 5 minutes, which is considered abnormal. An antinuclear factor test was positive. No anti-DNA antibodies were present. However, she had positive antibodies to SSA (Ro) and SSB (La). These findings of keratoconjunctivitis with the association of antibodies to Ro (SSA) and La (SSB) support the diagnosis of primary Sjögren's syndrome.

Previous pregnancies. She had three previous normal term deliveries. Her first child is doing well; however, her second child had a congenital dislocation of the hip and her third child was diagnosed as having acute myelocytic leukemia at the age of 1.

Present pregnancy. The present pregnancy was unremarkable except for occasional dryness of the eyes and the throat and one episode of upper respiratory tract infection which resolved rapidly with antibiotic treatment. However, a chronic nonproductive cough persisted for 6 weeks. An initial abdominal scan showed a normal gross fetal anatomy. Fetal echocardiogram showed a normal fetal heart; however, the atrial and ventricular rates were varying between 60 and 70 bpm. The fetal M-mode echocardiography showed a complete heart block. The pregnancy was then monitored by weekly abdominal ultrasound for fetal growth, for fetal ventricular sizes and function, and for evidence of fetal heart failure, namely, fetal pericardial effusion and enlarged umbilical vein diameter. At 41 weeks gestation she presented at 8 cm of dilatation and was delivered precipitately of a healthy female infant, weighing 2900 gm and with Apgar scores of 7 and 8. The blood pressure of the infant was in the range of 50/25 mm Hg, and the electrocardiogram showed a complete atrioventricular block at a rate of 50 to 60 bpm. The echocardiogram was found to be normal. The infant remained bradycardiac and began having premature ventricular contractions for which she had a transfemoral pacemaker placed. Subsequently she developed necrotizing enterocolitis which rapidly resolved on a regimen of antibiotics and parenteral hyperalimentation. The infant improved over the next few days and was discharged at the age of 22 days of life.

Comment

Congenital complete heart block, which has an incidence of 1/20,000 live births, can occur in anatomically abnormal hearts in which there is an interruption of the conducting system. Antinuclear antibodies, which are known to cross the placenta, may cause congenital heart block in a mother with collagen disease.[1]

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There is a family of circulating autoantibodies directed against components of the cell nucleus. Antibodies directed against extractable nuclear antigens can be found in systemic lupus erythematosus and other collagen diseases. Anti-SSA (Ro) anti-SSB (La), anti-RNP, anti-PMI, anti-Sm, anti-Sci-70, and anti-DNP are other such antibodies. Anti-Ro (SSA), anti-La (SSB), anti-RNP, anti-PM-1, anti-Sm, anti-Sci-70, and anti-DNP are frequently found in patients with primary Sjögren's syndrome. More specifically, the anti-SSA (Ro) antibody is a marker for risk of congenital complete heart block.[2] It is important to note that anti-Ro (SSA) alone does not produce a positive result for antinuclear antibody on conventional tests; a special test procedure is needed that requires the laboratory to be notified of the specific test wanted. In the patients with positive results for antinuclear antibody and negative results for systemic lupus erythematosus, an anti-Ro test should be requested. The presence of antibodies to Ro(SSA) in the maternal serum may result in permanent damage of the conducting system in infants, and this serves as a unique marker for the risk of isolated congenital complete heart block. Serial fetal echocardiography avoids an inappropriate obstetric intervention and premature delivery of such infants.

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Failure of exogenous prostacyclin to change placental and fetal blood flow in preeclampsia

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Seven patients with acute preeclampsia and six with superimposed preeclampsia were infused intravenously with incremental doses of prostacyclin (up to 8 ng/min/kg during 80 minutes). Prostacyclin infusion was accompanied by significant decreases in maternal blood pressure and consistent rises in maternal plasma or urinary 6-keto-prostaglandin $F_{1\alpha}$, but it caused no changes in maternal or fetal pulse rate or uterine contractility. Moreover, prostacyclin did not change the placental and umbilical blood flow, which were measured before and at the end of infusion. All women experienced facial flushing and two complained of headache during infusion. There was no difference in prostacyclin effects between women with acute or superimposed preeclampsia. These results may be taken as evidence that intravenous prostacyclin is not a specific therapy to increase placental or umbilical blood flow in preeclampsia. (AM J OBSTET GYNECOL 1985;151:661-5.)

Key words: Preeclampsia, prostacyclin, placental blood flow

There is much evidence to suggest that preeclampsia is accompanied—primarily or secondarily—by deficient generation of vasodilatory and antiaggregatory prostacyclin in maternal and fetoplacental tissue.[1-3] This may lead to generalized vasoconstriction, platelet activation, and even to reduced blood flow in the umbilical vessels.[4] Therefore much hope has been focused on the possible use of prostacyclin as a treatment of preeclamptic pregnancies, and indeed, exogenous prostacyclin decreased blood pressure in preeclamptic women.[5, 6] However, the fetal complications in preeclampsia are evidently a result of the inadequacy of placental blood flow.[7, 8] Thus it is conceivable that if prostacyclin has a specific therapeutic effect on preeclampsia, it should increase the blood flow in the placenta and/or fetus.

We therefore studied the effect of exogenous pros-

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Table I. Clinical characteristics as well as maternal blood pressure and heart rate before and at the end of prostacyclin infusion

Diagnosis	Case No.	Week of pregnancy	*Maternal blood pressure*				*Mean arterial pressure*		*Maternal heart rate*	
			Systolic		*Diastolic*					
			Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Superimposed preeclampsia	1*	35	157	151	91	80	102	91	78	78
	2*	36	172	177	112	103	129	119	87	97
	3	32	161	156	113	102	121	117	80	96
	4*	36	140	138	95	80	114	98	70	75
	5	32	148	129	98	71	113	88	78	71
	6*	37	141	134	91	70	105	88	108	90
Mean			153	147	100	84†	114	100†	84	85
± SE			4.9	7.3	4.1	6.0	4.1	5.8	5.4	4.6
Severe acute preeclampsia	7*	34	141	142	94	80	109	94	96	94
	8	28	144	123	107	73	120	83	73	83
	9*	28	165	167	107	106	128	114	82	92
	10	38	149	147	98	94	112	112	86	87
	11	33	157	153	103	93	123	113	58	72
	12	36	149	141	86	74	108	97	73	70
	13	31	160	147	101	94	119	116	61	66
Mean			152	146‡	99	88‡	117	104‡	76	81
+ SE			3.3	5.0	2.8	4.6	2.8	4.8	5.1	4.2
Total group		Mean	153	145‡	100	86§	116	102§	79	82
		± SE	2.8	4.9	2.3	3.6	2.4	3.9	3.8	3.1

*Treatment by clonidine before the study.
†$p<0.01$.
‡$p<0.05$.
§$p<0.001$.

tacyclin on the placental and fetal blood flow in human preeclampsia.

Material and methods

Thirteen women with preeclampsia were studied with the approval of the local committee of ethics between 28 and 38 weeks of gestation (Table I). Seven patients had been admitted to hospital because acute severe preeclampsia (blood pressure repeatedly ≥160/110 mm Hg and proteinuria ≥1.0 gm/day). Six women had already been hypertensive before pregnancy, but the blood pressure increased to 160/110 mm Hg during pregnancy, and proteinuria ensued. All patients were treated with bed rest and six patients with clonidine (150 to 300 μg/day) before the study (Table I), and blood pressure levels decreased from those at admission. The deliveries took place 1 to 13 days after the study abdominally (n = 9) or vaginally (n = 4). One newborn infant (Case 9) with a birth weight of 850 gm and gestational age of 30 weeks died at the age of 2 days because of respiratory distress syndrome. She was delivered by cesarean section, which was done for maternal indications (uncontrollable rise in blood pressure, proteinuria >10 gm/day). The other newborn infants, whose birth weights ranged from 980 to 2840 gm, were in good condition at delivery and survived.

Prostacyclin (epoprostenol), synthesized by The Upjohn Co. (Kalamazoo, Michigan) and formulated by the Wellcome Foundation (Beckenham, England) was dissolved in glycine buffer, pH 10.5, and infused intravenously by means of four incremental doses of 1, 2, 4, and 8 ng/min/kg for 20 minutes. The infusion was started at 10 AM, and 30 minutes before and during the infusion the women lay in 15-degree left laterally tilted position.

The intervillous blood flow was measured before and at the end of prostacyclin infusion by means of an intravenous xenon 133 isotope clearance method.[9] The result was expressed in milliliters of blood per minute per 100 ml of the intervillous space. For this method it is essential that the placenta lies on the anterior wall of the uterus, and this was the case in our subjects.

The umbilical vein blood flow was measured by a combination of real-time ultrasonography and pulsed Doppler ultrasound.[10] Briefly, the Doppler transducer was fixed at a 50-degree angle to the real-time transducer, which was adjusted on the maternal abdomen to lie parallel to the umbilical vein of the fetus. We used

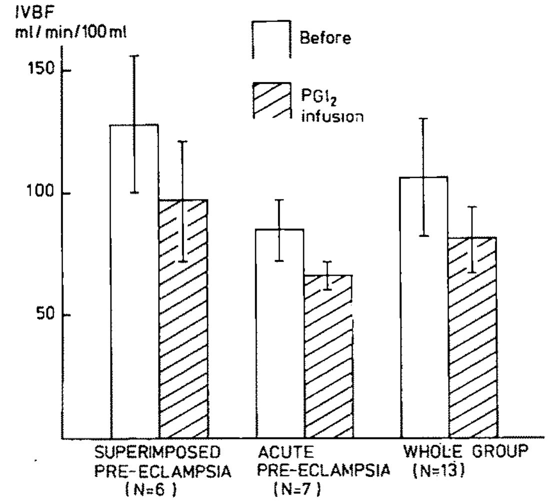

Fig. 1. Mean levels (±SE) of intervillous blood flow *(IVBF)* before and at the end of prostacyclin infusion in the whole group and the two subgroups.

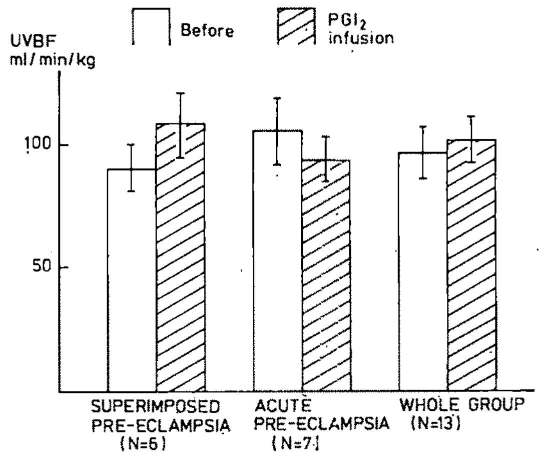

Fig. 2. Mean levels (±SE) of umbilical vein blood flow *(UVBF)* before and at the end of prostacyclin infusion in the whole group and the two subgroups.

the mean velocity of the blood in the umbilical vein and the vein diameter to calculate blood flow. The flow was expressed in milliliters of blood per minute per kilogram of fetal weight, which was estimated from the biparietal and body diameters at the time of flow measurement.[11]

The maternal blood pressure and pulse frequency were recorded at 3-minute intervals with Doppler equipment (Dinamap, Critikon Ltd., Broadlands, Tampa, Florida). Fetal heart rate and uterine activity were monitored constantly with external cardiotocography (Hewlett-Packard 8030A, Palo Alto, California).

Blood samples were collected into ice-cold heparinized tubes containing indomethacin (10 μmol/L, final concentration) before and 30 and 80 minutes after the start of infusion as well as 90 minutes after its end. The plasma was separated and used for the radioimmunoassay of 6-keto-prostaglandin $F_{1\alpha}$ (a breakdown product of prostacyclin).[12] Two 4-hour urine samples from the mothers were collected as well, one before the start of prostacyclin infusion and the other from the onset of infusion. A 10 ml urine sample was acidified to pH 3.0 with a 1N hydrochloric acid and passed through the octadecylsilyl silica cartridge (Sep-Pak$_{18}$ Cartridges, Waters Associates Inc., Milford, Connecticut), which was prewashed successively with 5.0 ml of ethanol, water, and 0.1N acetic acid.[13] The cartridge was then washed with 10% ethanol (5.0 ml) and petroleum ether (5.0 ml). The 6-keto-prostaglandin $F_{1\alpha}$ adsorbed was eluted in 2.0 ml of ethylacetate and evaporated to dryness under a nitrogen stream. The sample was further purified with high pressure liquid chromatography (M-740, Spectra-Physics Inc., Santa Clara, California), with use of a Sherisorb 5 μm ODS II column (Phase Separations Ltd., Industrial Estate, Queensferry, CLWD, England) and a mobile phase of water/acetonitrite/acetic acid (69.95:30.0:0.05) with a flow rate of 2 ml/min. 6-Keto-prostaglandin $F_{1\alpha}$ eluted at 3 minutes and separated totally from the other prostanoids. The fraction containing 6-keto-prostaglandin $F_{1\alpha}$ was collected, evaporated to dryness, and dissolved in phosphate-buffered saline solution with 0.1% of gelatine. Two different size aliquots, both in duplicate, were taken for the radioimmunoassay.[12] The result was expressed as nanograms of 6-keto-prostaglandin $F_{1\alpha}$ per 4-hour urine sample.

The results were subjected to the *t* test for paired observations and regression analysis (Spearman).

Results

Intervillous and umbilical vein blood flow. Prostacyclin infusion did not change intervillous blood flow in acute or superimposed preeclampsia (Fig. 1). In contrast, the intervillous blood flow in the whole group before prostacyclin infusion (106.4 ± 24.7 ml/min; mean ± SE) fell slightly during the infusion (81.0 ± 13.7 ml/min), although the fall was not significant ($p > 0.05$). The individual changes in intervillous blood flow during prostacyclin infusion were unrelated to the preinfusion intervillous blood flow. Also the umbilical vein blood flow was unaffected by prostacyclin infusion in the subgroups or in the total series of the patients (Fig. 2). The individual changes in umbilical vein blood flow during prostacyclin administration were not in correlation with the preinfusion umbilical and intervillous blood flow.

Maternal hemodynamics. The diastolic and mean

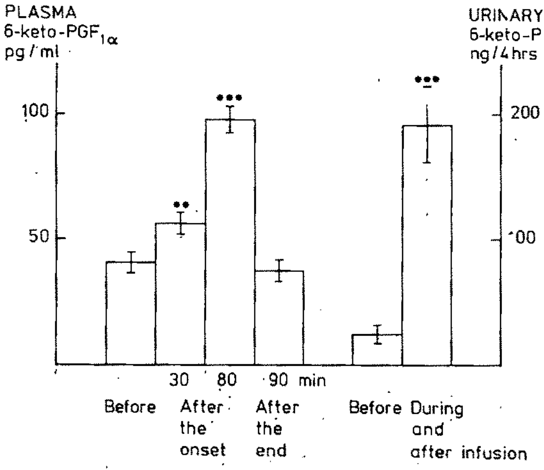

Fig. 3. Plasma and urinary 6-keto-prostaglandin $F_{1\alpha}$ (mean ± SE) before, during, and after prostacyclin infusion during late pregnancy. *Asterisks* indicate significant differences in comparison with the preinfusion value: ** = $p < 0.01$; *** = $p < 0.001$.

arterial pressure (mean arterial pressure = diastolic pressure + ⅓ pulse pressure) fell during prostacyclin infusion and also in women with acute or superimposed preeclampsia (Table I). This effect was already seen at the infusion rate of 1 ng/min/kg. The maternal heart rate did not change (Table I). Also, the patterns of fetal heart beat and uterine contractility were unaffected by prostacyclin.

All women experienced neck and facial flushing, which necessitated the lowering of the prostacyclin dose in Case 1. The subjects in Cases 6 and 10 complained of headache, and in Case 3 of restlessness, which also necessitated a temporal decrease in prostacyclin dose.

Plasma and urinary 6-keto-prostaglandin $F_{1\alpha}$. The prostacyclin infusion was accompanied by marked rises in plasma 6-keto-prostaglandin $F_{1\alpha}$, which disappeared, however, within 90 minutes after the infusion (Fig. 3). Also, the urinary 6-keto-prostaglandin $F_{1\alpha}$ excretion increased about 10 times during and following prostacyclin infusion. The individual rises in plasma or urinary 6-keto-prostaglandin $F_{1\alpha}$ were unrelated to the intervillous and umbilical vein blood flow.

Comment

The prostacyclin infusion up to 50 ng/min/kg is known to decrease blood pressure and to increase pulse rate in men[14-16] and in nonpregnant women.[17] We infused prostacyclin (up to 8 ng/min/kg) into pregnant women and found significant decreases in maternal blood pressure but no change in maternal or fetal pulse rate or in uterine contractility. Considering the specificity by which prostacyclin infusion increases pulse rate in nonpregnant subjects even before the fall of blood pressure,[3, 14-17] it was astonishing that the pulse rate did not rise in our pregnant volunteers. This may imply that the chronotropic mechanism becomes more or less tolerant to the effect of exogenous prostacyclin during pregnancy, perhaps as a result of increased generation of endogenous prostacyclin, which is characteristic for pregnancy. It remains to be seen whether this "tolerance" can be overcome by increasing prostacyclin doses, but such a study is not ethically sound at the present time.

In view of the instability of prostacyclin in physiologic pH and perhaps its more rapid metabolism in the pregnant than in the nonpregnant state, one may argue that the doses of prostacyclin were too small and perhaps never reached the placenta and/or fetus in concentrations high enough to exert a physiologic effect. However, this is unlikely, because prostacyclin is much more stable in the presence of albumin resembling the physiologic condition (half-life of about 60 minutes)[18] than in pure buffer solution (half-life of about 2-3 minutes). Furthermore, judging from comparable rises in plasma 6-keto-prostaglandin $F_{1\alpha}$ following prostacyclin infusion in nonpregnant women[19] and in pregnant women, as seen in this work, one may conclude that at least the hydrolysis of exogenous prostacyclin to 6-keto-prostaglandin $F_{1\alpha}$ does not differ between the nonpregnant and pregnant state. Therefore we suppose that prostacyclin reached the placenta in our subjects, but we do not know if it crossed it to the fetus.

We determined placental and umbilical blood flow with the methods that have been widely used in clinical research.[20, 21] These measurements, although still under some criticism in their reliability in absolute terms[22, 23] are most useful, for example, when relative flow changes in response to a drug treatment are being studied. In this work we infused prostacyclin in such doses that clear effects in maternal vasculature and plasma and urinary 6-keto-prostaglandin $F_{1\alpha}$ were seen. In view of prostacyclin deficiency in preeclampsia[1-3] and the initial optimism regarding the treatment of these complications with prostacyclin,[4-5] we expected to see increases in intervillous blood flow and/or umbilical vein blood flow during prostacyclin infusion. Further support for this expectation came from the data of Clark et al.,[24] who demonstrated a rise in uterine blood flow following prostacyclin infusion in pregnant sheep. However, prostacyclin infusion did not change the fetoplacental circulation in our subjects. Thus our human data seem to be in accordance with the results obtained from sheep models when maternal prostacyclin infusion led to increased cotyledonary resistance with decreased placental blood flow despite increased flow rate in nonplacental uterine areas.[25, 26]

In conclusion, intravenous infusion of prostacyclin decreases blood pressure in preeclamptic pregnancies. This may lend further support to the role of prostacyclin deficiency in this complication. Nevertheless, an infusion of pharmacologic doses of prostacyclin did not increase placental or umbilical blood flow. A more prolonged treatment with intravenous or oral prostacyclin analogues when they will be available could perhaps prove more effective than the short-term infusion of prostacyclin.

We are grateful to Dr. John O'Brady of Wellcome Co. for the supply of prostacyclin.

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CURRENT INVESTIGATION

Analysis of the human fetal skeleton and organs with xeroradiography

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We have used xeroradiography to study normal and abnormal fetuses including some with anencephaly, hydrocephalus, spina bifida, osteogenesis imperfecta (type IV), Jeune syndrome, radial aplasia, thanatophoric dysplasia, and Pena-Shokeir syndrome. Xeroradiography images the lines of ossification and epiphyses in great detail, shows ossification, and reveals abnormalities that alter bone modeling as seen in Jeune syndrome, osteogenesis imperfecta (type IV), and thanatophoric dysplasia. This technique can be used successfully to examine soft tissues and organs. It can also be used in combination with contrast materials to identify the lateral ventricles, the cardiovascular, gastrointestinal, urinary, and respiratory systems, and the cavities (pleural and peritoneal) of the fetal body. (AM J OBSTET GYNECOL 1985;151:666-70.)

Key words: Fetus, bone, xeroradiography, skeleton, ultrasonography

Xeroradiography is a technique that uses roentgen rays to image soft tissues. It has been used extensively in analysis of the human breast. Because of its high resolution of tissues with low mineral content, we use it to analyze the structure of the skeleton and organs of stillborn infants and fetuses that were spontaneously or voluntarily aborted.

The fetal skeleton has provoked the curiosity of researchers for a long time. The first account we have been able to find of a systematic analysis is the book *Osteogenia Foetuum*, written by Kerchringii[1] in 1717. This study was followed by others.[2-4] Cruikshank et al.[5] described the appearance of the ossification centers in the human fetus. Alizarin stain of the fetal skeleton has been used in humans and animals to analyze the development of bones[6].

When a spontaneous abortion or stillbirth occurs, it is important to examine the embryo or fetus carefully to determine the cause of death and the consequences for future reproduction. In cases of repeated spontaneous abortions or stillbirths, a genetic cause may be detected by examining the fetus. X-ray imaging has been recommended as part of the fetal examination.[7,8]

Here we describe the results of the use of xeroradiography in 66 fetuses sent to the Genetics Laboratory for study to demonstrate its high resolution and usefulness in the analysis of the human fetal phenotype because it reveals details not obtainable as clearly by other methods.

Material and methods

Fetuses aborted (spontaneously and voluntarily) are normally sent for phenotypic, cytogenetic, and biochemical analysis to the Genetics Laboratory. Conventional x-ray films are part of our routine examination. The last 66 of them (some normal and some affected by different conditions) were studied by xeroradiography (some by both methods). The cases of voluntary termination had been preceded by prenatal diagnosis, including ultrasonography and amniocentesis.

Anterior/posterior (Figs. 1 and 2) xeroradiographs and conventional x-ray images were obtained as soon after receipt of the fetus as possible. A complete autopsy was performed and tissue samples were obtained for culture and cytogenetic analysis. At times bones were removed and xeroradiographed (Figs. 3 and 4). When the autopsy was performed sectionally, xeroradiographs of the fetal sections were obtained. Contrast media were used to delineate the cardiovascular system, the lateral ventricles of the brain, the spinal canal, the

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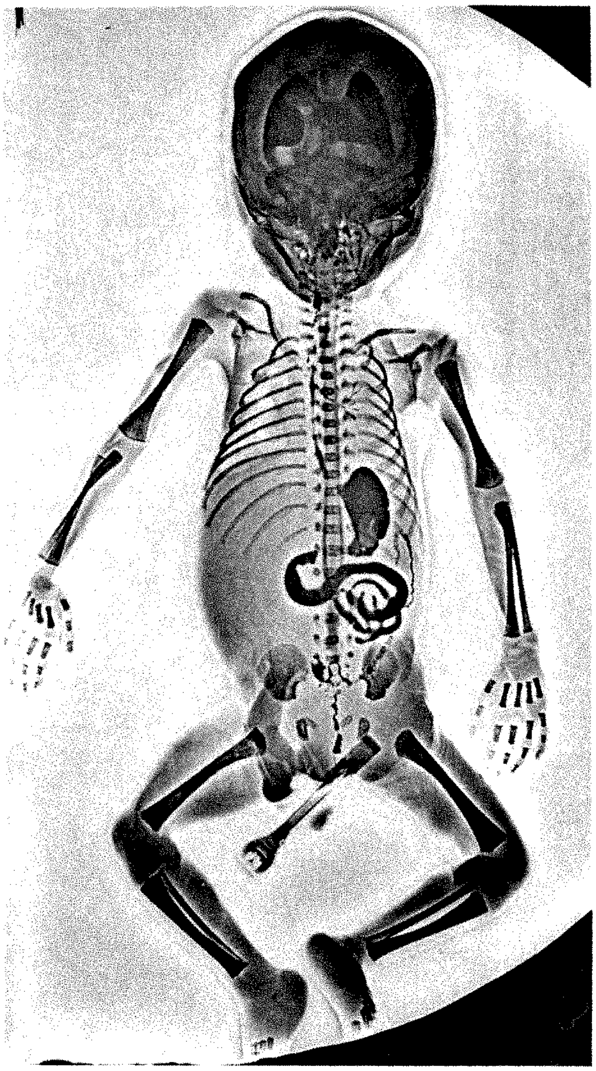

Fig. 1. Anterior/posterior xeroradiograph of a 21-week fetus. Note the detailed image of bone structure. Delineated by the contrast media are the ventricular system and the medulla and medullar canal (filled with contrast media). Also shown are the gastrointestinal tract (esophagus, stomach, intestine, and part of the left bronchial tree) and the thickness of the skin and subdermal tissue.

gastrointestinal tract (Figs. 1, 5, and 6), the urinary tract, and the bronchial tree (Fig. 5). A Picker x-ray instrument was used with the mammography fitting at 200 mA, 1½ seconds, and varying from 38 to 48 kV, depending upon fetal size. Both positive and negative xeroradiographic images were obtained. Since xeroradiography produces mirror images, it is important to invert the pictures so that the organs are localized in their normal position.

Results

This method produces detailed images of different bone structures including metaphyses (Figs. 1 to 6) and epiphyses (Figs. 3 and 4) because of the edge enhance-

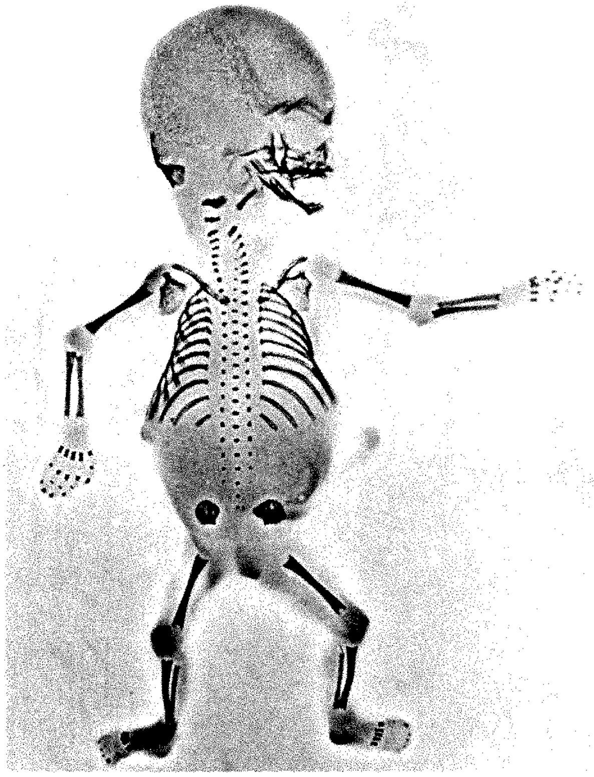

Fig. 2. Anterior/posterior view of a 12-week fetus. Note the resolution of the fetal skeleton that is not visible in this detail on conventional x-ray films and is most noticeable on the skull bones.

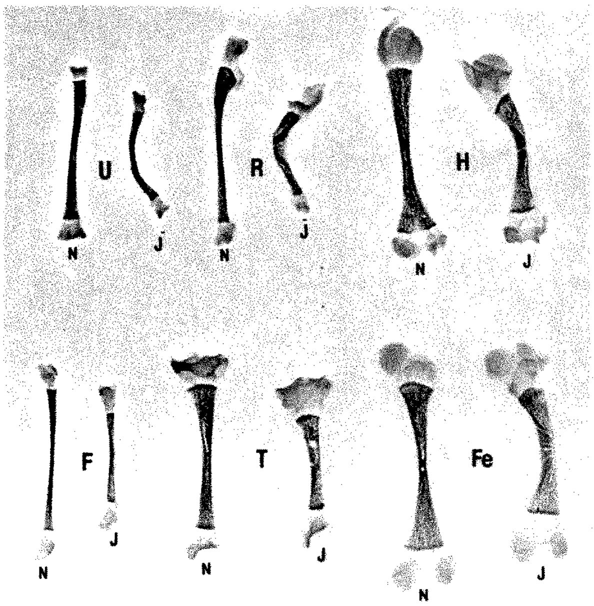

Fig. 3. Bones of two fetuses at 20 weeks of gestation, one affected by Jeune syndrome (*J*) and the other normal (*N*). Note the differences in the shape (Jeune syndrome bones are shortened and bowed) and in the structure of the endochondral and perichondral bone. Note the epiphyses and growth plate. *U*, Ulna; *R*, radius; *H*, humerus; *Fe*, femur; *T*, tibia; *F*, fibula.

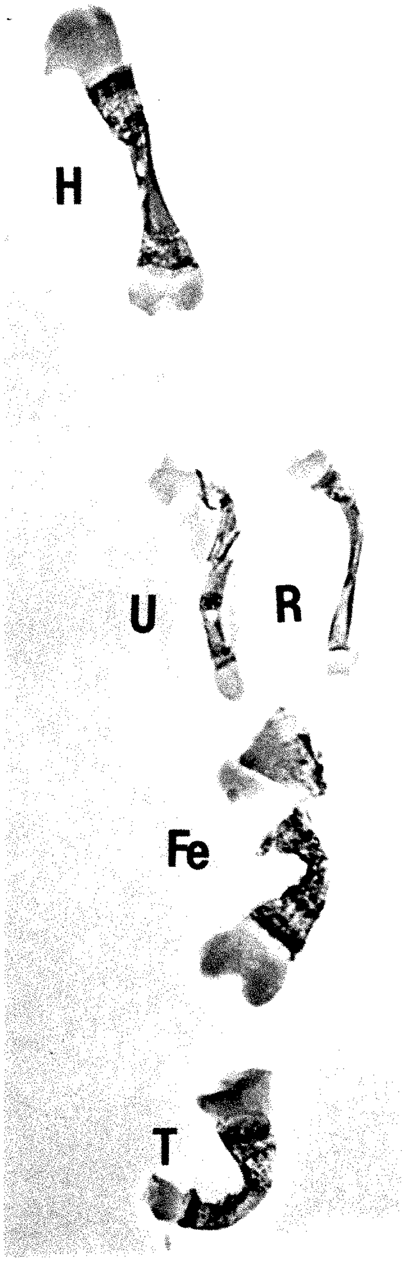

Fig. 4. Bones [humerus *(H)*, ulna *(U)*, radius *(R)*, femur *(Fe)*, tibia *(T)*] of a fetus at 19 weeks of gestation affected by osteogenesis imperfecta type IV. Note the abnormal shape of metaphyses and epiphyses that detach easily, the abnormal structure of the endochondral and perichondral bones, and the fractures.

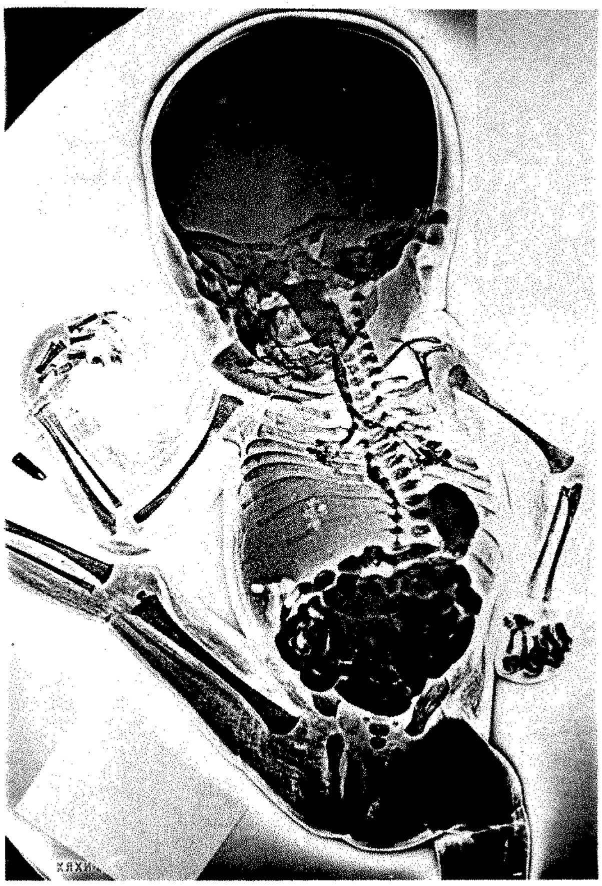

Fig. 5. Anterior/posterior view of a stillborn infant affected by the Pena-Shokeir syndrome. Note the positioning of the extremities and the oropharynx, trachea, and gastrointestinal tract, which is delineated by contrast media. The joints are fixed (arthrogryposis multiplex). The ribs are abnormally shaped and positioned; also delineated are the skin and the subdermal tissue and muscular bundles, most noticeable on the right thigh and leg.

ment qualities of xeroradiography. For best results the bones should be removed from the fetal body. The quality of the image is better than that produced with conventional x-ray films. It also allows for the early visualization of unmineralized bones at 10 to 12 weeks of gestation when the skeleton is barely visible on x-ray films (Fig. 2).

This method has been used to analyze fetuses with major defects detected ultrasonographically: (1) Conradi disease at 19 weeks, ultrasonographically characterized by short bones with metaphyseal stippling: (2) Pena-Shokeir syndrome detected by ultrasonography that showed absent movements, contractures, and hypoplastic maxilla; (3) VACTERL syndrome (Vertebral anomalies, Anal atresia, congenital Cardiac disease, Tracheoesophageal fistula, Renal anomalies, Radial dysplasia, and other Limb defects) at 35 weeks; (4) short cord syndrome at 18 and 20 weeks, with extremely short cord and abnormal lower spine, absent vertebral and iliac bones, and abnormal abdominal wall; (5) ureteral obstruction at 33 weeks, detected by ultrasonography because the abdomen was filled with fluid and there was an empty bladder with an abnormally dilated ureter and duodenal atresia; (6) hydrocephalus and spina bifida at 22 weeks—an ultrasonographic examination showed dilated ventricles due to stenosis of the aqueduct of Sylvius and spina bifida; and (7) anencephaly. All of these conditions and the signs cited were diagnosed in utero by ultrasonography. Three examples are shown in this paper to illustrate the results obtained: Jeune syndrome at 17 weeks (Fig. 3), osteogenesis imperfecta at 19 weeks (Fig. 4), and Pena-Shokeir syndrome at 34 weeks (Fig. 5). Most of the fetuses (41) were normal by xeroradiography, external examination, and autopsy.

Fig. 3 shows bones of a normal fetus designated *N* and those of the fetus affected by Jeune syndrome. All long bones of the Jeune syndrome fetus show a narrow diaphysis with very little or almost absent endochondral bone and prominent perichondral bone; this area corresponds to the center of angulation showing the excess of perichondral bone on the concave side.

Xeroradiographs of bones of a fetus with osteogenesis imperfecta (Fig. 4) show complete absence of the ossification rays of normal endochondral bone and almost complete absence of perichondral bone; they also show fractures and bowing. The epiphyses detach easily from the metaphyses. Ossification is spotty.

Xeroradiography also images the axial skeleton (Figs. 1, 2, 3, 5, and 6) in greater detail than conventional x-ray films, with visualization of the ossification centers and trabeculae in (1) the vertebrae, the transverse and spinal processes, and the vertebral bodies; (2) the skull bones; (3) the scapulas; and (4) the iliac bones. Images obtained with contrast media allow for the examination of fetal organs such as the lateral ventricles and the cardiovascular, gastrointestinal, urinary, and respiratory systems. Because of their small size, these organs are very difficult to examine adequately by anatomic dissection. Xeroradiography will detect abnormalities not recognizable by other means, such as an obstruction of the bronchial system, the esophagus, or the gastrointestinal and urinary tracts.

Positive xeroradiographs were obtained for all fetuses; negative xeroradiographs were taken from only three of them. The quality of the positive image was by far better than that of the negative one, which is not routinely used. Several attempts have been made to visualize soft tissue including the heart, muscular bundles, and others; so far we have not been successful with fetuses in the first and second trimesters. On the basis of results obtained when third-trimester fetuses were analyzed (Figs. 5 and 6), the technique appears to be promising; at this stage, the stomach, intestine, skin, muscular bundles, and oropharynx are seen with the same technique used to visualize the skeleton.

Comment

When a dead fetus is delivered, a very important issue is to analyze the parents and the fetus as thoroughly and completely as possible in order to find the probable cause. The information obtained is used to assess the risk of recurrence and to counsel the family.

When a pregnancy is interrupted after prenatal diagnosis (ultrasonography and amniocentesis) because the fetus is abnormal, a complete examination with accurate documentation should be done, including pictures, microscopic examination, and x-ray visualization of the fetal skeleton. Recently, we decided to use xeroradiography not only because of its sharp and clear resolution of fetal bone structure and because it shows details not visible by eye, conventional x-ray films, or histologic analysis but also because it demonstrates the organization of the ossification centers, the trabeculae, and the structure of the epiphyses (Figs. 3 and 4). It also differentiates the endochondral bones from the perichondral bones (Figs. 3, 4, and 5).

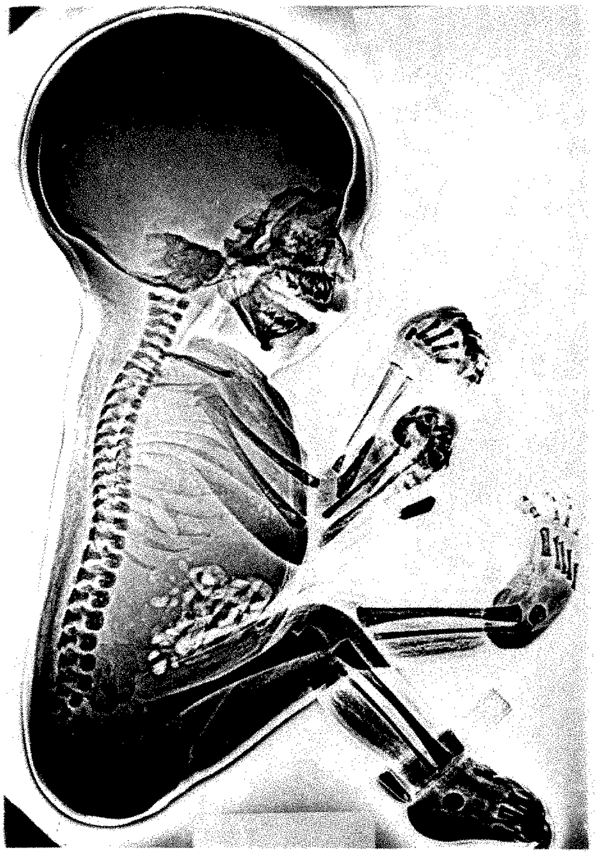

Fig. 6. Lateral view of the same fetus before contrast media were used, demonstrating hypoplasia of the midface and of the mandible. Note the oropharynx delineated by air, including the upper part of the trachea. The spine appears shorter than normal. Note the abnormal shape of the vertebral bodies and the position of the extremities. The ossification of the bones is clearly noticeable. The stomach and intestine are visualized without contrast media. The muscular bundles are also visible on both legs.

The defects on the diaphyseal structure of the bones of the fetus with Jeune syndrome (Fig. 3), osteogenesis imperfecta type IV (Fig. 4) and thanatophoric dysplasia are interesting examples of the possibilities of this technique. The fetus with Jeune syndrome (Fig. 3) appeared to have a reduced amount of endochondral bone with very narrow diaphyses suggesting a failure in the process that normally follows absorption of this type of bone. The fibulae were not bowed; it is known that the fibula has a different system of ossification which is regulated by direction of the nutrient artery of the bone.[9] The fibulae were severely shortened and thinned when compared with a normal bone. This observation, not documented before in the human fetus, may not be unique of Jeune syndrome but indicates that there are major defects in the way the embryonal endochondral bone is absorbed, the formation of the medullar canal, and how the perichondral bone develops. In osteogenesis imperfecta (Fig. 4) a more profound defect in the structure of the bone is suggested by the xeroradiographic images characterized by clumping of the ossification rays that are normally linear following the main axis of the bone. In osteogenesis imperfecta the bones are severely bowed and the perichondral bone is almost completely absent.

Another advantage of this technique is its ability to visualize organs and soft tissues inside the fetal body (Figs. 1, 5, and 6). The combination of this technique with the use of contrast media injected through the arterial, ventricular, bronchial, gastrointestinal, and urinary systems and into different cavities (peritoneal and pleural) produces detailed views of the system and thus enhances our ability to detect abnormalities of the fetal organs and systems.

In the third trimester of gestation, some other structures are revealed in the fetus: the intestine, the oropharynx, the skin, the subdermal tissue, and the muscular bundles (Figs. 5 and 6). Refinement of the technique will allow for a more sophisticated view of the fetal organs and tissues.

In cases of hydrocephalus it allows for the determination of the different types, especially when the aqueduct is stenotic and does not allow the fluid to flow to the spine as it normally does when injected into the ventricle.

We propose that this technique be used for the analysis of fetuses that are received for genetic or anatomic/pathologic analyses and for the analysis of normal and abnormal fetal bones, organs, and tissues, with or without contrast media, to enhance the completeness of the examination of the human fetus.

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Endotoxemia in the neonatal lamb

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Although gram-negative sepsis is a major cause of neonatal morbidity and mortality, our understanding of endotoxemia in the neonate has been hampered by the lack of experimental models. Previous studies have suggested neonatal hyporesponsiveness to endotoxin. We studied unanesthetized neonatal lambs which had been exposed to the environment prior to study. These animals demonstrated the classic early phase changes of endotoxemia including pulmonary hypertension which was dependent upon prostanoid production. This model allows further studies of endotoxemia in the neonate. (AM J OBSTET GYNECOL 1985;151:671-4.)

Key words: Endotoxemia, neonate, sepsis

Although antibiotic therapy has significantly reduced the impact of pediatric infections, gram-negative sepsis is a major cause of neonatal morbidity and mortality.[1] Current understanding of endotoxic shock in the adult has relied heavily upon observations in experimental models. The pathophysiology of this syndrome in the neonatal animal, however, has received little attention. Since developmental differences in response to the effects of gram-negative sepsis may exist,[2, 3] we studied the short-term effects of *Escherichia coli* endotoxin injection in the neonatal lamb model. As prostaglandins play an important role in the manifestations of endotoxemia in the adult ewe, we further investigated the role of prostaglandins E and F and inhibition of cyclooxygenase on this response.

Material and methods

Studies were conducted on mixed-bred lambs of either sex of less than 7 days of age (range, 0.5 to 6.0 days; mean, 2.8 days). The lambs were maintained on a farm with their mothers and transported on the day of the procedure.

The cervical region was infiltrated with 4 to 6 mg of Xylocaine and the common jugular vein and carotid artery were exposed. A flow-directed balloon catheter (Fr. 5, Swan-Ganz, Edwards Laboratories, Santa Ana, California) was inserted into a jugular vein and advanced into the pulmonary artery. Correct catheter placement was determined by observing the characteristic pressure tracings and was confirmed at necropsy. Early trials demonstrated that repeated balloon inflation for determination of mean capillary wedge pressure or rapid injection of iced saline led to catheter displacement or unreliable recordings. Therefore only measurements of mean pulmonary arterial pressures were performed. The carotid artery was cannulated with a No. 18-gauge catheter via the same exposure. Mean pulmonary and systemic arterial pressures were measured with pressure transducers (Statham P37B, Statham Co., Oxnard, California) and recorded on a multi-channel recorder (Hewlett-Packard, Rockville, Maryland).

Endotoxin was obtained from commercial sources (*E. coli* 0 111:B4 lipopolysaccharide, Difco Laboratories, Detroit, Michigan) and was prepared on the day of the procedure with pyogen-free 0.9% sodium chloride used to yield a final concentration of 0.5 mg/ml.

All blood samples were collected from peripheral arteries via indwelling catheters and were replaced isovolumetrically with sterile saline solution. Arterial P_{O_2}, pH, P_{CO_2}, and base excess were determined with an analyzer calibrated at 37.5° C (IL 813, Instrumentation

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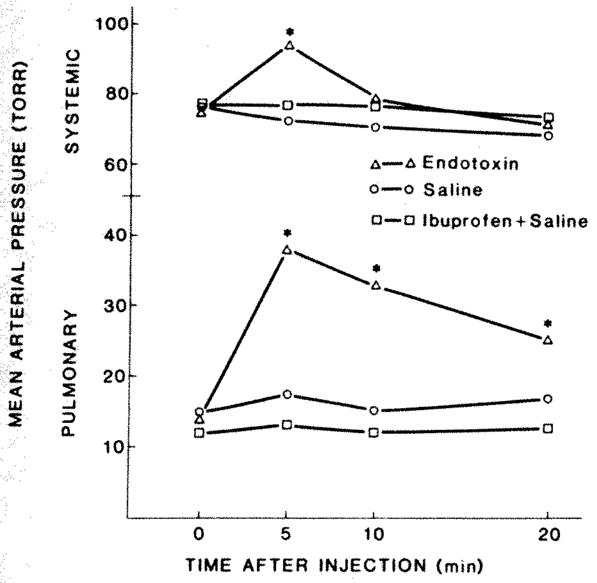

Fig. 1. Mean systemic and pulmonary arterial pressures prior to (time 0) and following injection of endotoxin (1 mg/kg) or saline solution (2 ml/kg). $^*p < 0.05$, Student's paired *t* test, difference from time 0.

Laboratories, Lexington, Massachusetts) and were corrected for hematocrit. Leukocyte and platelet counts were determined manually from ethylenediaminetetraacetic acid–containing samples and differential counts were made from smears. Samples for determination of plasma levels of prostaglandins F and E were collected into heparinized tubes maintained at 0° C containing indomethacin to yield a final concentration of 10 μg/ml. Tubes were centrifuged at 3° C within 10 minutes of collection and stored at −70° C until analysis.

Systemic plasma levels of prostaglandins F and E were determined via radioimmunoassay following extraction with ethyl acetate as previously reported.[4] All samples were assayed in duplicate and results were corrected for extraction loss. The intra-assay coefficient of variation was 8% and the interassay coefficient of variation was 11%. Results were expressed as picograms of prostaglandin F or E per milliliter of plasma.

Three groups of animals were studied. Group 1 (n = 4) received 1 mg/kg of endotoxin. Group 2 (n = 4) received 1 mg/kg of endotoxin 30 minutes after receiving 10 mg/kg of sterile sodium ibuprofen (The Upjohn Company, Kalamazoo, Michigan). Group 3 (n = 3) received pyogen-free sterile sodium chloride (vehicle), 2 ml/kg. All solutions were injected into the superior vena cava of the lamb.

Results

Mean arterial pressures. Mean pulmonary and systemic arterial pressures prior to and following injections are illustrated in Fig. 1. Lambs receiving endotoxin alone demonstrated a rapid rise in mean pulmonary arterial pressure with a subsequent decline toward baseline values during the 20-minute observation period. This group also demonstrated a transient increase in mean systemic arterial pressure which was evident only at 5 minutes after injection.

Table I. Systemic arterial Po_2 prior to (time 0) and following injection of 1 mg/kg of *E. coli* endotoxin (groups 1 and 2) or 0.9% saline solution (group 3) into neonatal lambs

	Po_2 (torr, mean ± SEM)			
Group	*0*	*+5 min*	*+10 min*	*+20 min*
1	72 ± 3	44 ± 3*	49 ± 4*	51 ± 6*
2	86 ± 12	83 ± 14	93 ± 12	84 ± 16
3	84 ± 5	80 ± 6	74 ± 6	84 ± 6

Group 1 (n = 4): endotoxin, 1 mg/kg; group 2 (n = 4): ibuprofen, 10m/kg plus endotoxin, 1 mg/kg; group 3 (n = 3), 0.9% saline solution, 2 ml/kg.

$^*p < 0.01$, differences from time 0, Student's paired *t* test.

Lambs receiving either saline or endotoxin following ibuprofen did not demonstrate any significant change in pulmonary or systemic arterial pressures.

Systemic arterial pH and blood gases. Values of systemic arterial Po_2 are listed in Table I. Lambs receiving endotoxin alone demonstrated a fall in Po_2 following injection with a subsequent rise toward preinjection values. No significant changes were noted in the groups receiving either saline solution or endotoxin following ibuprofen.

None of the groups demonstrated any significant changes in systemic arterial pH, Pco_2, or base deficit.

Prostaglandin levels. Systemic arterial levels of immunoreactive prostaglandin F and prostaglandin E are listed in Table II. Baseline values for both prostaglandins were significantly lower in lambs receiving ibuprofen prior to sampling (group 2) than in groups 1 and 3. Lambs receiving endotoxin alone demonstrated significant rises in prostaglandins F and E at 5, 10, and 20 minutes following injection. No significant change in prostaglandin levels were noted in lambs receiving endotoxin following ibuprofen or saline solution injection.

Peripheral platelet and leukocyte counts. Systemic arterial platelet counts, leukocyte counts, and percentage of neutrophils in the three groups are listed in Table III. Lambs receiving endotoxin alone or endotoxin following ibuprofen demonstrated significant decreases in platelet counts, leukocyte counts, and percentage of neutrophils. No significant changes were noted in lambs receiving saline solution.

Table II. Systemic arterial levels in prostaglandins F and E prior to (time 0) and following injection

	Arterial level (pg/ml, mean ± SEM)			
Group	*0*	*+5*	*+10*	*+20*
Prostaglandin F				
1	189 ± 52	1418 ± 232*	956 ± 164*	979 ± 414*
2	34 ± 2†	43 ± 12	38 ± 7	33 ± 2
3	157 ± 44	148 ± 45	195 ± 21	162 ± 43
Prostaglandin E				
1	168 ± 42	790 ± 198*	725 ± 141	904 ± 244*
2	52 ± 23†	53 ± 23	55 ± 26	88 ± 14
3	124 ± 32	141 ± 42	155 ± 44	139 ± 42

Group 1 = endotoxin, 1 mg/kg; group 2: ibuprofen, 10 mg/kg, plus endotoxin, 1 mg/kg; group 3, 0.9% saline solution, 2 ml/kg.

*$p < 0.01$, difference from time 0, Student's paired *t* test.

†$p < 0.01$, difference from groups 1 and 3.

Table III. Systemic platelet count, leukocyte count, and percentage of neutrophils prior to (time 0) and following injection

Group	*0 min*	*+5 min*	*+10 min*	*+20 min*
Platelets ($\times 10^6/mm^3$)				
1	762 ± 174	410 ± 49*	421 ± 43*	579 ± 115*
2	972 ± 148	842 ± 57†	770 ± 92*	705 ± 64*
3	694 ± 121	688 ± 151	694 ± 125	663 ± 93
Leukocytes ($\times 10^3/mm^3$)				
1	4.32 ± 1.23	0.96 ± 0.22*	0.96 ± 0.20*	0.90 ± 0.15*
2	4.55 ± 1.03	1.05 ± 0.13	0.98 ± 0.14	1.10 ± 0.20*
3	3.83 ± 0.50	3.83 ± 0.49	4.03 ± 0.78	3.93 ± 0.89
Polymorphonuclear leukocytes (%)				
1	41 ± 6	0.8 ± 0.6*	1.0 ± 0.2*	4.0 ± 0.6*
2	48 ± 8	2.2 ± 0.6*	2.7 ± 1.1*	3.7 ± 1.2*
3	51 ± 6	50 ± 7	51 ± 2	48 ± 3.4

Values are mean ± SEM.

*$p < 0.01$, difference from time 0, Student's paired *t* test.

†$p < 0.05$, difference from time 0, Student's paired *t* test.

Comment

In addition to advancing our knowledge of the pathophysiology of endotoxin shock, laboratory models have allowed controlled investigations of therapeutic regimens.[4, 5] These investigations have documented the role of the prostaglandins as well as other vasoactive substances in the early phases of endotoxin shock.[6, 7]

In contrast to these studies in adult animals, little information is available on the effects of endotoxin in the neonate. The effects of endotoxin on the fetus are largely indirect and mediated by changes in uterine blood flow and maternal hyperthermia.[8, 9] Infusions of endotoxin directly into the fetal lamb fail to produce alterations in blood pressure or circulating levels of prostaglandins.[2, 10]

Several studies have provided evidence that this fetal hyporesponsiveness is continued into the neonatal period. Bech-Jansen et al.[2] were unable to produce shock in neonatal lambs with doses several times higher than that required in ewes. Similar findings have been noted in regard to the febrile response[3] and Schwartzman phenomenon[11] in newborn rabbits.

In the current investigation the neonatal lamb was chosen in view of the extensive literature available on the effects of endotoxin in the ewe. Endotoxin was injected at doses similar to those required to produce consistent pulmonary changes in the adult ewe. In contrast to the above-cited reports, there were dramatic responses in pulmonary arterial pressure, systemic Po_2, and circulating levels of prostaglandins. As in the ewe, the acute pulmonary effects of endotoxin could be prevented by prior administration of ibuprofen, strongly suggesting that they are dependent upon the production of prostaglandins or other cyclo-oxygenase products.

The reason for difference between our observations and those of Bech-Jansen et al.[2] in the neonatal lamb are not apparent but several differences in the experimental designs exist. First, in our studies, lambs were not anesthetized whereas small amounts of pentobarbital sodium were used in the prior study. Perhaps more important, in our studies lambs were born on a farm and were therefore exposed to bacterial antigens in the environment. The neonates studied by Bech-Jansen et al. were delivered by hysterotomy, immediately intubated, and studied within 10 to 20 minutes. Several studies have suggested that prior exposure to bacterial antigens may be important in the pathogenesis of the early phase of endotoxemia.[12, 13] This exposure would not have been possible in animals studied soon after hysterotomy and may account for the lack of response noted in that study.

In summary, our results demonstrate that, under the conditions of our study, the neonatal lamb is responsive to endotoxin injection. This response includes pulmonary hypertension, decreases in white blood cell and platelet counts, and elevation of circulating levels of prostaglandins. This observation allows the use of this model for future studies of the pathogenesis and therapy of endotoxemia in the neonate.

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Reproductive tract abnormalities in female mice treated neonatally with tamoxifen

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Immature female mice of the NMRI strain were treated with 20 μg/day of tamoxifen or vehicle for the first 3 days after birth. At 90 days of age, vaginas, uteri, and ovaries were examined histologically. A benign epithelial change, vaginal adenosis characterized by abnormal growth of columnar epithelium with glandular structures, was found in all of the tamoxifen-treated mice. Anatomic anomalies such as hypospadias, cervical hypoplasia, uterine hypoplasia, and absence of corpora lutea also were commonly observed. Such abnormalities were not found in the control mice. (AM J OBSTET GYNECOL 1985;151:675-8.)

Key words: Mouse, tamoxifen, vaginal adenosis, hypospadias, hypoplasia

Tamoxifen is a nonsteroidal derivative of triphenylethylene and is capable of interfering with the actions of estrogens. Therefore, the antiestrogen tamoxifen has now been widely used for the purpose of endocrine therapy of estrogen receptor–positive human breast cancer.[1] On the other hand, an estrogen-like effect of tamoxifen has also been reported. For example, in female neonatal rats, tamoxifen treatment induces an increase of uterine weight[2] and luminal epithelial cell height within a few days.[3] Furthermore, it should be noted that abnormal changes such as atrophic ovaries and uteri and squamous metaplasia of the oviducts occurred in adult rats that received tamoxifen at shortly after birth.[4] It is well known that the recent increase of abnormalities of the reproductive tract in women is linked to exposure in utero to the synthetic estrogen diethylstilbestrol.[5] The estrogen-like property of tamoxifen may cause abnormalities similar to those induced by diethylstilbestrol. Tamoxifen has been used in an attempt to induce ovulation in sterile women.[1, 6] Therefore, studying the effect of tamoxifen on the developing reproductive tract is significant. The mouse model has proved to be very valuable for studying normal and abnormal development of the reproductive tract.[7-11] In this paper, we describe the effects of tamoxifen on the developing reproductive tract in the NMRI strain of mouse.

Material and methods

NMRI mice (F15-5) were obtained from the University of California in 1983 (Dr. G. R. Cunha, San Francisco, California) and were maintained by brother-sister mating in our laboratory in an air-conditioned room with periods of 14 hours of light and 10 hours of dark. Pregnant mice were put into separate cages to give birth to their pups. Fifteen female pups from five mothers were given three daily subcutaneous injections of 20 μg of tamoxifen (Sigma Chemical Co., St. Louis, Missouri) in 0.05 ml of saline solution, starting within approximately 24 hours after birth. As controls, 10 female pups from three mothers were given three daily subcutaneous injections of 0.05 ml of saline solution. All mice were put to death by means of ether at 90 days of age. Vaginas, uteri, and ovaries were fixed in Bouin's solution, embedded in paraffin, sectioned transversely at 6 μm, and stained with hematoxylin and eosin.

Results

Abnormalities detected in reproductive organs of NMRI mice treated neonatally with tamoxifen are summarized in Table I. All of the reproductive tracts of tamoxifen-treated mice had two striking anatomic anomalies. First, "hypospadias" characterized by an abnormal urethral opening into the vaginal lumen with epithelial hyperplasia of the urethra was seen (Fig. 1, *A*); under the influence of tamoxifen, the separation of urethral and vaginal canals by mesenchymal layers was affected. In control mice, the epithelium of the urethra and vagina was surrounded by proper mesenchymal layers (Fig. 1, *B*). Second, a remarkable reduction in the size of the common cervical canal was observed (Fig. 2, *A*). In addition an abnormal appearance of the heterotopic columnar epithelial cells with many folds toward the underlying mesenchyme and formation of

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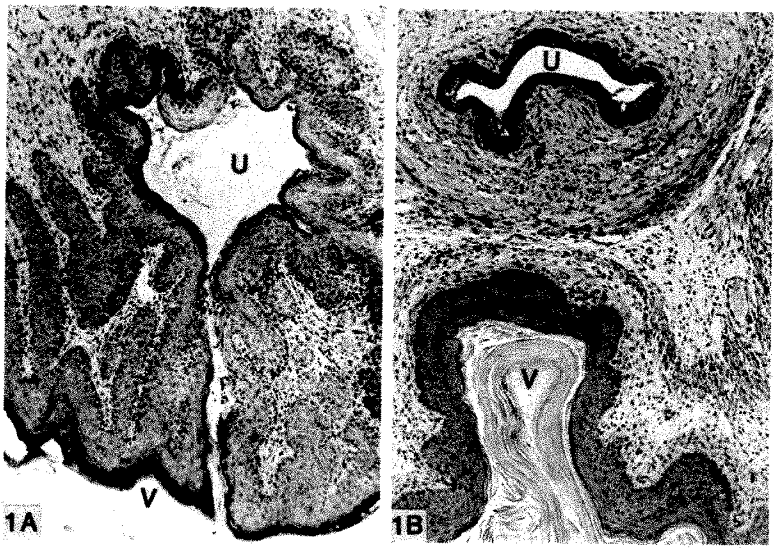

Fig. 1. Cross sections through vaginas *(V)* and urethras *(U)* of 90-day-old NMRI mice. (Original magnification ×110.) *A*, Tamoxifen-treated mouse. Note opening of urethra into vagina and epithelial hyperplasia. *B*, Control mouse. Note both vagina and urethra have normal mesenchyme.

Table I. Abnormalities of the reproductive tract in NMRI mice treated neonatally with tamoxifen

Treatment	*No. of mice*	*Hypospadias*	*Vaginal adenosis*	*Cervical hypoplasia*	*Uterine atrophy*	*Corpus luteum failure*
Tamoxifen*	15	15	15	15	15	15
Saline solution	10	0	0	0	0	0

*Mice were injected for 3 consecutive days with 20 μg/day of tamoxifen. All mice were killed at 90 days of age.

adenosis-like glandular structures were observed in the regions of the upper vagina, vaginal fornix, and cervix in all of the neonatally tamoxifen-treated mice (Fig. 2, *A* and *B*). No columnar cells were present in any of the same regions of the control mice (Fig. 2, *C*).

The overall structure of the uteri of all tamoxifen-treated mice appeared to be atrophic with sparse uterine glands (Fig. 3, *A*). However, the uterine epithelium of the mice was highly stimulated, with a pattern similar to that of control mice during the estrous cycle (Fig. 3, *B*). The thinner mesenchymal and muscular layers of the uteri of the tamoxifen-treated mice were attributed to atrophy of the uteri (Fig. 3, *A*). In addition, the ovaries of tamoxifen-treated mice were somewhat atrophic without formation of corpora lutea. No abnormalities were found in the uteri and ovaries of control mice.

Comment

The potential teratogenic and carcinogenic effects of exogenous estrogens on the developing reproductive tract in humans[5, 12, 13] and experimental animals[8-11] are now well established. The mouse reproductive tract has been identified as a good developmental model.[7] In the reproductive tracts of some older female mice that received estrogen during the fetal and/or neonatal period, neoplastic and preneoplastic changes such as adenocarcinoma, squamous cell carcinoma, and preneoplastic vaginal basal cell downgrowth have been observed.[10, 11] In the younger mice, a higher incidence of benign epithelial changes such as hyperplastic squamous vaginal epithelium and vaginal adenosis has been observed.[8, 9] Structural changes of the reproductive organs such as hypospadias, cervical hypoplasia, and failure of development of corpora lutea have been frequently observed throughout the life span of mice treated with estrogen shortly after birth.[9-11] The present results indicate that injections of tamoxifen into newborn NMRI mice can consistently elicit abnormalities of the reproductive tract that have morphologic patterns very similar to those induced by perinatal estrogen exposure. For example, in the mice treated neonatally with tamoxifen, heterotopic columnar epithelial cells were interspersed with the stratified squamous epithelium lining the regions of the upper vagina, fornix, and cervix. The columnar epithelium also formed downgrowths that resembled glandular structures. These lesions may be analogous to the adenosis that has been observed in diethylstilbestrol-exposed animals[9, 11] and humans.[6] We have no information about

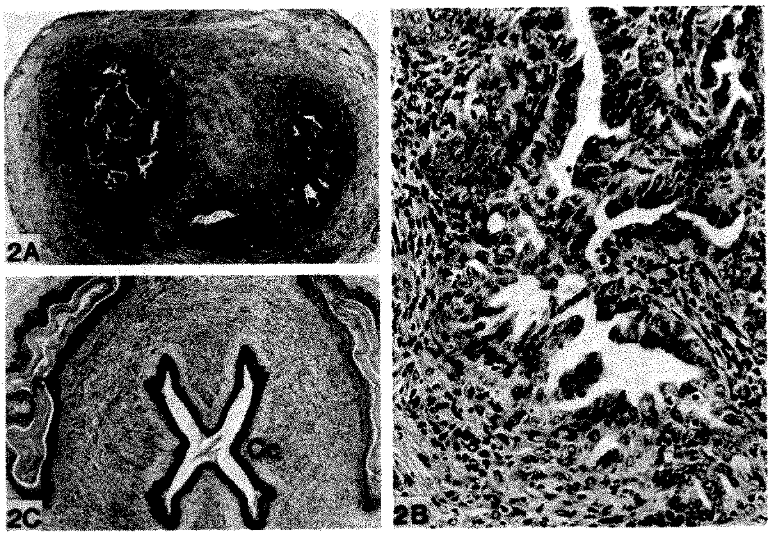

Fig. 2. Cross sections through common cervical canal *(Cc)* and vaginal fornices *(F)* of 90-day-old NMRI mice. *A* and *B*, Tamoxifen-treated mouse. *A*, Note tiny common cervical canal and glandular structures in fornices. (Original magnification ×30.) *B*, Enlargement of glandular structures. (Original magnification ×260.) *C*, Control mouse. (Original magnification ×30.)

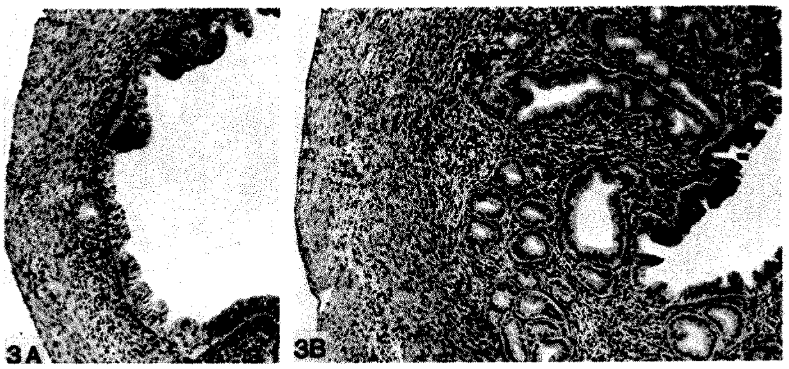

Fig. 3. Cross sections through uteri. (Original magnification ×110.) *A*, Tamoxifen-treated mouse. Note poor formation of mesenchyme and muscle. *B*, Control mouse.

the ultimate fate of adenosis in the tamoxifen-treated mice and have also little information about that in the diethylstilbestrol-treated mice, but signs of malignancy within the adenosis regions in some older mice after neonatal diethylstilbestrol exposure[11] suggest that some relationship may exist between the adenosis and malignancy. In addition, gross hypoplasia of the uterocervical canal was consistently observed in tamoxifen-treated mice. This is also a significant finding, because a similar abnormality has been reported in diethylstilbestrol-exposed mice[9, 10] and women.[5]

Although we have not eliminated the possibility of indirect effects of tamoxifen on the reproductive tract, it seems likely that tamoxifen directly affects the target tissues. Recent biochemical studies with radiolabeled hormones have demonstrated that tamoxifen prevents the binding of estradiol to estrogen target tissues,[14] while estradiol inhibits the binding of monohydroxytamoxifen to the rat uterus.[15] Therefore, the effects of tamoxifen on the reproductive tract are thought to be mediated by estrogen receptors in the tissues. The binding of tamoxifen to estrogen receptors may act as an estrogenic stimulation in the developing reproductive tract. Moreover, steroid autoradiographic analysis has demonstrated that both epithelial and mesenchymal cells of the reproductive tract of the mature female mouse possess nuclear estrogen-binding sites. However, only the mesenchymal cells at an early stage of development of the female reproductive tract contain nuclear estrogen-binding sites.[16] The lack of estrogen-binding sites within the epithelial cells of the reproductive tract at this stage suggests that tamoxifen affects epithelial cells via the mesenchymal cells. Such a possibility of a mesenchymal role is shown by urogenital tissue recombination studies.[16]

Tamoxifen is now widely used as an antitumor agent against human breast cancer.[1] Tamoxifen has also been used for induction of ovulation.[1, 6] Although the daily dose of tamoxifen in this study was about 10 times that for humans,[1] the administration period was only 3 days. On the other hand, for human therapy, especially for patients with advanced breast cancer, tamoxifen has been used for many months. Long-term or repeated exposure to tamoxifen may affect some normal estrogen receptor–positive tissues. The therapeutic use of tamoxifen in anovulatory women must be applied especially carefully because of the possible fetal exposure.

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Antibodies to the neutral glycolipid asialo ganglio-*N*-tetraosylceramide: Association with gynecologic cancers

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As part of our efforts to define subpopulations at increased risk for gynecologic malignancies, sera from 145 women were obtained prior to diagnosis and analyzed for antibody to asialo ganglio-*N*-tetraosylceramide. This neutral glycolipid is present on the surface of thymocytes and natural killer cells, and asialo ganglio-*N*-tetraosylceramide antibody has been shown in animals to block natural killer cell activity and promote tumor cell proliferation. With the use of an enzyme-linked immunosorbent assay and with a value of 2 SD above the mean for healthy women designated as the boundary for a positive response, antibody to asialo ganglio-*N*-tetraosylceramide was detected in only one of 30 (3%) healthy women, none of 16 pregnant women, none of 18 women with benign masses, and two of 24 (8%) women with microbial infections. All of the above samples that contained antibodies were barely over the 2 SD limit. In marked contrast, 19 of 35 (54%) women with gynecologic malignancies had asialo ganglio-*N*-tetraosylceramide antibodies, with positive values ranging to >10 SD above the control mean. Asialo ganglio-*N*-tetraosylceramide antibody was found in six of eight (75%) patients with cervical cancer, five of eight (63%) with endometrial cancer, and seven of 15 (47%) with ovarian cancer. Of the eight patients with Stage I gynecologic cancer at any site, five (62%) had asialo ganglio-*N*-tetraosylceramide antibodies. Four of 22 (18%) women with Hodgkin's disease also had antibodies, with values just exceeding 2 SD above control levels. The presence of these antibodies may contribute to an impaired immune surveillance system in these women and so increase their susceptibility to malignancy. (AM J OBSTET GYNECOL 1985;151:679-81.)

Key words: Antibody, neutral glycolipid, asialo ganglio-*N*-tetraosylceramide, gynecologic cancer

Although several risk factors have been identified, it is still unclear why certain women develop gynecologic malignancies and others do not. Recent evidence with other types of cancer have focused attention on the development of certain antibodies which can hinder immune surveillance mechanisms, thereby aiding tumor cell proliferation.

Asialo ganglio-*N*-tetraosylceramide (GMl) is a neutral glycolipid present on the surface of human,[1] rat,[2] and mouse[3] natural killer cells and to a lesser extent on thymocytes.[4] Natural killer cells are believed to function as a major component of the immune surveillance system since they can recognize and destroy malignantly transformed and virus-infected cells.[5] Injection of rats[6] or mice[7] with antibody to asialo GMl was shown to greatly reduce natural killer cell activity and enhance the likelihood of tumor cell proliferation in vivo. Similarly, patients with acquired immune deficiency syndrome, who have low natural killer cell activity and are prone to develop several malignancies,[8] were recently shown to possess antibody to asialo GMl.[9, 10]

Material and methods

Patients and sera. Blood was obtained prior to diagnosis from 123 women seen in the Department of Obstetrics and Gynecology at The New York Hospital and from 22 women diagnosed at Memorial Hospital as having Hodgkin's disease. After clotting, sera were collected by centrifugation and stored in aliquots at −20° C until used. Diagnoses were determined with the use of physical, microbiologic, and pathologic criteria whenever appropriate.

Asialo GMl antibodies. Antibodies to asialo GMl were determined by an enzyme-linked immunosorbent assay, as previously described.[9] Briefly, asialo GMl in chloroform : primary alcohol (3 : 1) was added to wells of a polyvinyl microtiter plate (1.2 μg per well) and placed under vacuum for 16 hours. The wells were then washed three times with phosphate-buffered saline

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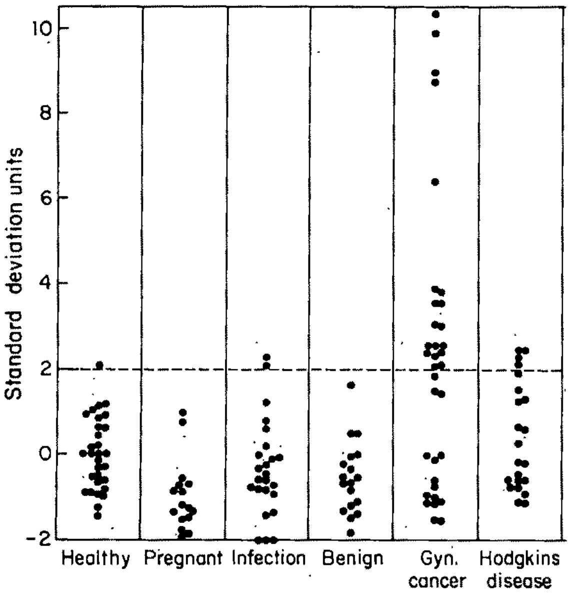

Fig. 1. Antibody to asialo GMl in women. Sera from 145 women were assayed for asialo GMl antibody by an enzyme-linked immunosorbent assay. The *dotted line* indicates 2 SD above the mean value obtained for healthy women.

(PBS)–0.05% Tween 20 and test sera were added for 120 minutes at room temperature. The wells were again washed and bound IgG was quantitated by determination of the optical density (OD) at 405 nm following sequential incubation with alkaline phosphatase-conjugated swine antihuman IgG (Medical Diagnostics, Hackensack, New Jersey) and the alkaline phosphatase substrate *p*-nitrophenyl phosphate. All samples were assayed in duplicate and the mean value was determined. Results were converted into standard deviation (SD) units by the following formula: SD units = OD (patient) − OD (control mean)/SD (controls). A value of 2.0 was designated as the lower boundary for specific reactivity.

Results

Asialo GMl antibodies in women. The reactivities of IgG from patients' sera with asialo GMl are shown in Fig. 1. Only one of 30 (3%) women in apparently good health and two of 24 (8%) women with infections were found to have asialo GMl antibody; the positive values were of borderline significance. Similarly, none of 16 pregnant women and none of 18 women with benign masses possessed this antibody. Thus, of 88 women without malignancy, only three (3.4%) sera yielded experimental values >2 SD above those of the control women. In women with nongynecologic cancer (Hodgkin's disease), four of 22 (18%) had asialo GMl antibody, but similar to the levels in the population with benign conditions, the values were just above the 2 SD limit.

In marked contrast to the above results, sera from 19 of 35 (54%) women with gynecologic cancers yielded asialo GMl antibody. Values ranging to >10 SD above the control level were observed. If a cutoff value of 3 SD above the control mean were to be chosen, then 11 of 35 (31%) gynecologic cancer sera and zero of 110 other sera would contain asialo GMl antibody.

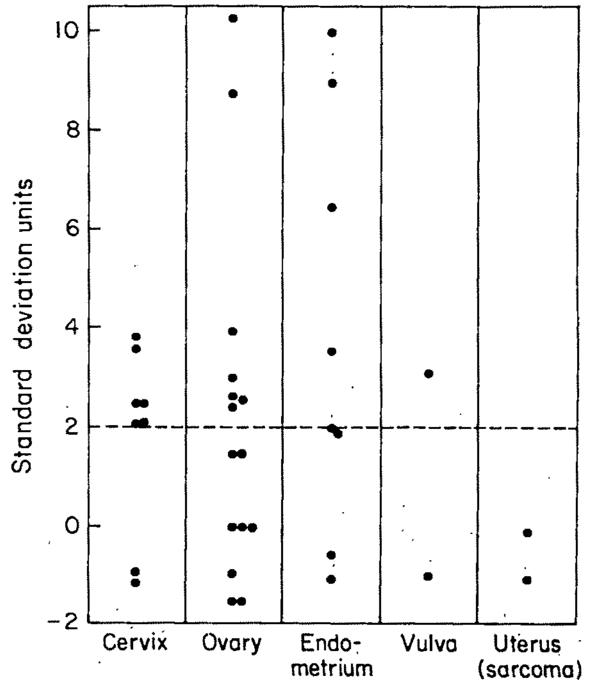

Fig. 2. Antibody to asialo GMl in gynecologic cancer patients. Sera from 35 women with gynecologic malignancies were assayed for asialo GMl antibody, as in Fig. 1.

Antibody to asialo GMl in gynecologic cancer. Asialo GMl antibody levels in the various gynecologic malignancies are shown in Fig. 2. Antibody was demonstrated in sera of six of eight (75%) with cervical cancer, five of eight (63%) with endometrial adenocarcinoma, seven of 15 (47%) with ovarian cancer, one of two with vulvar cancer, and none of two with uterine sarcoma. Values >6 SD above the control mean were recorded for sera from three endometrial and two ovarian cancers.

Information regarding the stage of the malignancy was available for 27 patients with ovarian, cervical, or endometrial cancer. The presence of asialo GMl antibody was not confined to any particular stage, being detected in five of eight (63%) Stage I, two of three (67%) Stage II, six of 11 (55%) Stage III, and one of five (20%) Stage IV malignancies (Table I).

Comment

Among women, the presence of antibody reactive with asialo GMl appears to be a distinctive characteristic of patients with pelvic malignancies. The appearance of this antibody is not limited to women with advanced

Table I. Asialo GM1 antibody in gynecologic cancers

Stage	No. positive/total No.			All
	Ovarian cancer	*Cervical cancer*	*Endometrial cancer*	
I	0/1	2/2	3/5	5/8
II	–	2/3	–	2/3
III	3/8	2/2	1/1	6/11
IV	1/3	0/1	0/1	1/5

disease. Its detection in women with Stage I malignancy and its complete absence in women with benign tumors suggest the possible utility of asialo GMl antibody levels as an early serologic marker for cancer. This is an exciting possibility. To date, there has been no serologic test for women with endometrial or ovarian malignancy comparable to the Papanicolaou test for cervical malignancy. Since asialo GMl is a surface marker of natural killer cells[1-3] and thymocytes[4] and asialo GMl antibody abrogates in vivo natural killer cell activity in experimental animals,[6,7] it is possible that asialo GMl antibody plays a direct role in suppressing cellular immunity and decreasing the efficiency of destruction of nascent gynecologic neoplasms. Therefore, its presence may indicate an increased susceptibility to malignancy. A study to directly correlate asialo GMl antibody levels, natural killer cell activity, and lymphocyte functions is now in progress.

It is not clear why asialo GMl antibodies would appear specifically in association with gynecologic malignancies. There is an epidemiologic association between cervical cancer and sexual activity,[11,12] and most women with endometrial and ovarian cancers have been sexually active. With this background, it is intriguing to speculate on the possible role of spermatozoa in altering immune responses which can then predispose to malignancy. Most women do not produce antibodies to spermatozoa following intercourse. Even in situations such as vasectomy, where sperm antibodies are frequently produced, asialo GMl antibodies and other immune changes are not manifested.[10] However, under conditions not yet defined, exposure to spermatozoa can result in immunosuppression. Semen introduced into the rectums of healthy rabbits is followed by the appearance of antibodies to asialo GMl.[13] Exposure to spermatozoa has also been shown in some instances to result in suppression of the immune respone to T-lymphocyte–dependent antigens[13] and to the occurrence of antibodies cross reactive with T lymphocytes.[10] Perhaps in humans sexually transmitted herpesvirus, papillomavirus, or bacteria act as adjuvants to promote immune responses to spermatozoa, which lead to immunosuppression. This, in turn, would create more favorable conditions for both microbial replication and neoplastic cell proliferation. Alternatively, asialo GMl antibodies could arise in women by immunization with male lymphoid cells which were present in the ejaculate in increased numbers because of infection. Regardless of the eventual mechanisms, these data highlight the necessity of further exploring the relationship between infection, exposure to spermatozoa, immunosuppression, and malignancy.

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Cardiac output distribution and uteroplacental blood flow in the pregnant rabbit: A comparative study

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This study presents data on cardiac output distribution and uterine and placental blood flows in pregnant rabbits under chronic steady-state conditions. Ten litters and 67 fetuses were studied at 29 days of gestation, by means of radioactive microspheres. Five nonpregnant female animals were also studied for comparison. Mean cardiac outputs were 747.16 ± 55.7 and 613.80 ± 63.76 ml/min in the pregnant and nonpregnant states, respectively. In the pregnant animals, uterine and mammary blood flows were 6.7% ± 0.7% and 5.1% ± 0.5% of cardiac output, respectively. Within litters, the highest placental blood flows occurred at the ovarian and vaginal ends of the uterine horn. Placental blood flow per gram of fetus was 0.106 ± 0.008 ml · min^{-1} · gm^{-1}. A comparison with analogous data in the guinea pig and sheep demonstrates that toward the end of pregnancy placental blood flow per gram of fetus is approximately 2.5-times higher in sheep than in rabbits and guinea pigs. Expressed as a percentage of cardiac output, near-term uterine blood flow is significantly less in rabbits than in guinea pigs and sheep, whereas mammary blood flow is significantly higher. These interspecies differences are related to differences in placental structure, fetal/maternal mass ratio, and maturity at birth. (AM J OBSTET GYNECOL 1985;151:682-6.)

Key words: Pregnancy, cardiac output, uteroplacental blood flow

There are major differences among mammals in the factors that may influence the response of the maternal organism to pregnancy, for instance, differences in body weight, fetal metabolic demands, length of gestation, placental structure, number of fetuses, and degree of maturity at birth. Therefore, comparative data are required for a general understanding of the physiology of gestation and the appropriate use of laboratory animals for an understanding of the physiologic adaptations to human pregnancy.

Cardiac output and its distribution as well as placental blood flows have been measured in several species by means of the microsphere technique. The use of these data for comparative purposes is limited by differences in the state of the preparation (anesthetized versus conscious animals), by differences in methodology, and by the absence of relevant information (for instance, placental and fetal weights) in some studies. In the present series of experiments, cardiac output distribution and blood flow to the reproductive organs were measured in conscious nonpregnant rabbits and in rabbits at the twenty-ninth day of gestation, with the primary purpose of collecting information that could be used for a comparison with similar data in sheep and guinea pigs.

Material and methods

Ten pregnant and five nonpregnant New Zealand White rabbits weighing between 3.9 and 5.3 kg were studied. For the pregnant animals, operation was performed at 22 days' gestation. Anesthesia was induced with 150 mg of ketamine and 45 mg of xylazine given intramuscularly. Polyvinyl catheters (0.34 mm internal diameter and 0.23 mm external diameter) were placed in the left ventricle and distal aorta. Placement of the left ventricular catheter was confirmed by monitoring the pressure tracing as the catheter was advanced from the right carotid artery. The distal aorta catheter was placed through the right femoral artery and advanced approximately 5 cm. All catheters were secured with suture and surgical adhesive (Eastman 910). Both catheters were tunneled subcutaneously to an area between the scapulae and secured in a plastic cap sutured to the skin. Patency of the catheters was maintained by daily flushing with a heparin-saline solution (200 units per milliliter). After the operation the animals were kept in the laboratory in cages constructed to minimize reaction to daily laboratory routine and given food and water freely. Recovery to the preoperative level of food

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Table I. Comparison of organ weight and blood flow in pregnant and nonpregnant rabbits

Organ	*Weight (gm) (mean ± SEM)*		*Blood flow (ml·min⁻¹) (mean ± SEM)*	
	Pregnant (n = 10)	*Nonpregnant (n = 5)*	*Pregnant (n = 10)*	*Nonpregnant (n = 5)*
Carcass	2457.1 ± 164.9	2466.0 ± 45.7	311.9 ± 39.3	305.8 ± 69.1
Skin	534.1 ± 27.41	587.6 ± 23.8	47.93 ± 6.51	55.96 ± 14.16
Gut	153.7 ± 5.85	151.0 ± 6.0	121.7 ± 9.7	92.39 ± 16.94
Liver	138.3 ± 10.42	120.1 ± 7.4	7.40 ± 1.44	5.61 ± 0.98
Pancreas	2.90 ± 0.38	2.22 ± 0.38	3.24* ± 0.34	1.64 ± 0.40
Spleen	1.71 ± 0.21	2.42 ± 0.29	4.10 ± 0.90	8.26 ± 1.83
Kidneys	28.04 ± 1.47	24.08 ± 1.34	78.67 ± 5.87	74.62 ± 14.30
Heart	11.88 ± 0.65	10.77 ± 0.58	19.74 ± 2.18	18.42 ± 1.17
Brain	8.16 ± 0.27	8.51 ± 0.56	7.37 ± 0.70	7.57 ± 1.46
Eyes	6.56* ± 0.24	5.03 ± 0.37	6.19 ± 0.81	3.86 ± 0.86
Adrenals	0.66 ± 0.08	0.69 ± 0.07	0.27 ± 0.02	0.27 ± 0.04
Lungs	25.16 ± 3.33	22.07 ± 2.85	40.30 ± 5.39	23.84 ± 8.66
Subtotal	3368.28 ± 93.10	3400.49 ± 53.20	648.81 ± 49.9	598.17 ± 63.05
Ovaries	1.33* ± 0.09	0.67 ± 0.14	3.89* ± 0.77	0.62 ± 0.09
Mammaries	103.76* ± 8.73	18.78 ± 3.5	38.04* ± 4.36	2.89 ± 0.77
Uterus plus cervix	425.20* ± 51.70	19.98 ± 3.0	56.42* ± 5.70	12.12 ± 2.58
Total	3989.57* ± 107.58	3439.92 ± 53.81	747.16 ± 55.7	613.80 ± 63.76

*Significantly different from nonpregnant value at $p < 0.1$.

intake was achieved approximately 3 days after operation.

Microsphere technique. All animals were studied on the seventh postoperative day, which, in the pregnant animals, corresponded to the twenty-ninth day of gestation. Radioactive "carbonized" microspheres (New England Nuclear) labeled with either cobalt (15 or 25 μm in diameter) or tin (42 μm in diameter) were injected through the ventricular catheter while an integrated reference sample was withdrawn from the femoral arterial catheter by means of a calibrated pump. Six pregnant and five nonpregnant animals were injected with a mixture of 42 and 15 μm microspheres. Four pregnant animals received a mixture of 42 and 25 μm microspheres. The rate of withdrawal was 2.59 ml/min and the total number of microspheres injected ranged between 200,000 and 300,000. The microspheres were suspended in 10% Dextran and saline solution warmed to body temperature. The animals remained in the special cages during the study with minimal exposure to laboratory personnel. At the termination of the experiment, the animals were put to death and all organs dissected. Catheter placement was confirmed. All organs except skin and carcass were counted in total in a Nuclear-Chicago gamma counter equipped with a 3-inch NAI crystal. Ten aliquots of skin and carcass were used after homogenization. The uterus was dissected into placental and nonplacental portions and counted separately. Placentas were separated from the uterus by blunt dissection. All individual placentas were weighed and counted. The relative position of each site of implantation in the uterine horn was calculated by assigning a value of 100 to the length of the uterine horn from the ovarian to the cervical end and the implantation sites were arbitrarily assigned equivalent spacing. For example, in a horn containing a total of four implantation sites, the sites were assigned positions 20, 40, 60, and 80. Organ blood flows were calculated as previously described.[1]

Results

To ascertain whether the mixing of the 42 μm microspheres in arterial blood was adequate for organ flow measurements, the distribution of microspheres to the right and left kidneys was examined in the 11 animals injected with a mixture of 42 and 15 μm microspheres. The right and left renal blood flows ($ml \cdot min^{-1} \cdot gm^{-1}$) measured by the 15 μm microspheres were well correlated ($r = 0.928$) and not significantly different (right, 2.60 ± 0.27; left, 2.74 ± 0.24; $p > 0.1$). The two renal flows measured by the 42 μmm microspheres were also well correlated ($r = 0.913$) and not significantly different (right, 2.85 ± 0.29; left, 3.06 ± 0.28; $p > 0.1$). The percentage of the injected microspheres that was trapped in the lungs was significantly higher for the 15 μm microspheres (15 μm microspheres: 5.87% ± 0.87%; 42 μm microspheres: 4.65% ± 0.75%; $p < 0.001$), indicating peripheral shunting of the smaller spheres.

Cardiac output distribution. Organ weights and blood flows in the pregnant and nonpregnant group are compared in Table I. The weights of the nonreproductive organs were similar in both groups. The reproductive organs (gravid uterus, mammaries, and

Table II. Placental and myoendometrial blood flows in 10 rabbits at twenty-ninth day of gestation

Animal No.	*No. of fetuses**	*Fetal weight (gm)*	*Placental weight (gm)*	*Myo-endometrial weight (gm)*	*Fetal weight (gm)*	*Placental blood flow (ml/min)*	*Myo-endometrial blood flow (ml/min)*	*Total uterine flow (ml/min)*
80-6	4 (1)	185	21.5	38.9	245	14.2	11.6	25.8
80-8	9 (1)	308	77.2	60.1	445	27.3	16.1	43.4
80-25	9 (1)	363	79.6	78.2	521	45.6	18.9	64.5
80-26	9 (1)	325	48.8	51.0	425	36.9	8.9	45.8
80-27	9 (0)	420	84.4	69.2	574	49.0	15.0	64.0
80-30	2 (6)	80	17.3	83.6	181	12.5	46.0	58.5
81-27	6 (3)	241	47.6	61.2	350	20.0	10.5	30.5
81-29	5 (0)	196	48.9	75.4	320	18.4	27.7	46.1
81-30	11 (0)	480	91.0	89.1	660	45.7	28.2	73.9
81-33	3 (4)	174	26.0	46.1	246	18.7	19.6	38.3

*The numbers in parentheses indicate the number of dead fetuses.

Table III. Comparison between near-term pregnant sheep (130 to 140 days' gestation), rabbits (29 days' gestation), and guinea pigs (58 to 67 days' gestation).

	*Sheep** *(n = 7)* *(mean ± SEM)*	*Rabbit* *(n = 10)* *(mean ± SEM)*	*Guinea pig†* *(n = 6)* *(mean ± SEM)*
Placental blood flow			
Per gram of fetus ($\text{ml} \cdot \text{min}^{-1} \cdot \text{gm}^{-1}$)	0.26 ± 0.02	0.106 ± 0.008	0.114 ± 0.02
Per gram of placenta ($\text{ml} \cdot \text{min}^{-1} \cdot \text{gm}^{-1}$)	2.8 ± 0.2	0.57 ± 0.05	1.65 ± 0.3
Fetal/maternal weight ratio	0.07	0.07 ± 0.01	0.25 ± 0.02
Uterine blood flow			
As % of cardiac output	15.7 ± 1.3	6.7 ± 0.7	12.8 ± 2.5
Mammary blood flow			
Per gram of organ ($\text{ml} \cdot \text{min}^{-1} \cdot \text{gm}^{-1}$)	0.30 ± 0.03	0.37 ± 0.05	0.33 ± 0.07
As % of cardiac output	2.05 ± 0.3	5.1 ± 0.5	1.4 ± 0.3

*Data from Rosenfeld (see reference 5).

†Data from Myers et al. (see reference 1). Data on mammary blood flow from Peeters et al. (see reference 4).

ovaries) weighed 491 gm more in the pregnant state and accounted for a significantly higher body weight of the pregnant animals. The blood flows to ovaries, mammaries, and gravid uterus were all significantly higher in the pregnant group, representing a total increase of 83 ml/min over the nonpregnant level. Of this increase, a relatively large fraction (42%) was due to increased mammary flow. The mean sum of organ blood flows (cardiac output) was 133 ml/min higher in the pregnant group, reflecting in part the increased perfusion of the reproductive organs. The pancreas was the only nonreproductive organ showing a statistically significant increase in its perfusion rate.

Uterine and placental blood flows. Uterine and placental blood flows measured by means of 42 and 25 μm microspheres were not significantly different. By contrast, blood flows measured by means of the 42 μm microspheres in the placenta, the myoendometrium interposed between the sites of implantation, and the myoendometrium surrounding the conceptus were 10% ($p < 0.5$), 21% ($p < 0.1$), and 45% ($p < 0.01$), respectively, higher than the flows measured by means of the 15 μm microspheres. This finding indicates that a significant portion of the smallest spheres had escaped the uterine vasculature. Therefore, the 15 μm microsphere data were not used in the final calculation of flows. Uterine weight and blood flow data for the 10 pregnant animals are presented in Table II. The number of fetuses alive at the completion of the studies ranged from two to 11 per litter, and the combined fetal weight ranged from 80 to 480 gm. Placental blood flow (milliliters per minute) and fetal weight (grams) were linearly related: Placental flow = − 1.04 + 0.108 fetal weight ($r = 0.94$). Placental blood flow was 0.106 ± 0.008 ml/gm of fetus and 0.088 ± 0.006 ml/gm of combined fetal-placental weight. Blood flow to the myoendometrium ranged between 0.55 and 0.17 ml/gm with a mean of 0.303 ± 0.03 ml/gm. In the five animals with a large litter (nine to 11 fetuses) placental blood flow was 71% ± 4% of total uterine blood flow.

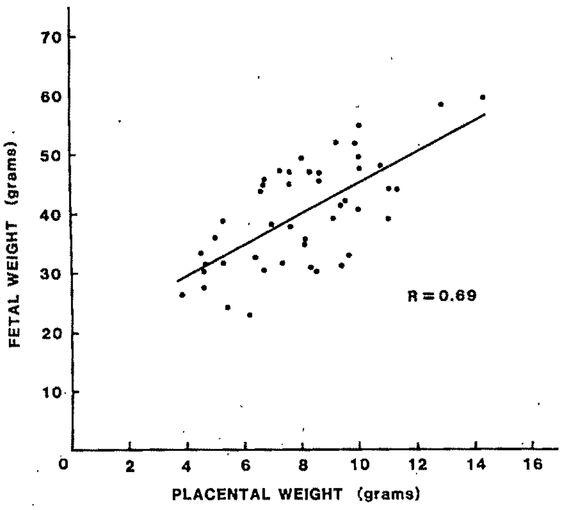

Fig. 1. Relationship of fetal weight and placental weight on day 29 of pregnancy in five rabbits, each carrying a large litter of nine to 11 fetuses.

Within each of the five large litters there was a positive correlation between fetal and placental weight, with a mean intercept of 14.2 ± 4.2 gm on the fetal weight axis (Y axis) and a mean slope of 3.4 ± 0.67. Regression analysis of the pooled data (Fig. 1) gave a similar result: Fetal weight = 19.1 + 2.6 × placental weight (n = 47, r = 0.69). Within each of these litters there was also a positive correlation between fetal weight and placental blood flow, with a mean intercept of 22.0 ± 4.5 on the fetal weight axis and a mean slope of 4.1 ± 0.96 gm/ml. The pooled data were described by the linear regression (Fig. 2): Fetal weight = 25.4 + 3.4 × placental flow (n = 47, r = 0.67).

The effect of fetal number on the magnitude of placental blood flow per fetus was examined by plotting blood flow to each placenta versus litter size. No significant correlation was found.

To examine the effect of site of implantation on placental blood flow, the blood flow to each placenta was expressed as a percentage of the mean flow in the uterine horn and plotted against the position in the horn. The greatest placental flows occurred at the ovarian and vaginal ends of the horn (Fig. 3). The smallest flows within a litter were more frequent in the middle of the horn, which was also the region having the highest incidence of dead fetuses (Fig. 3).

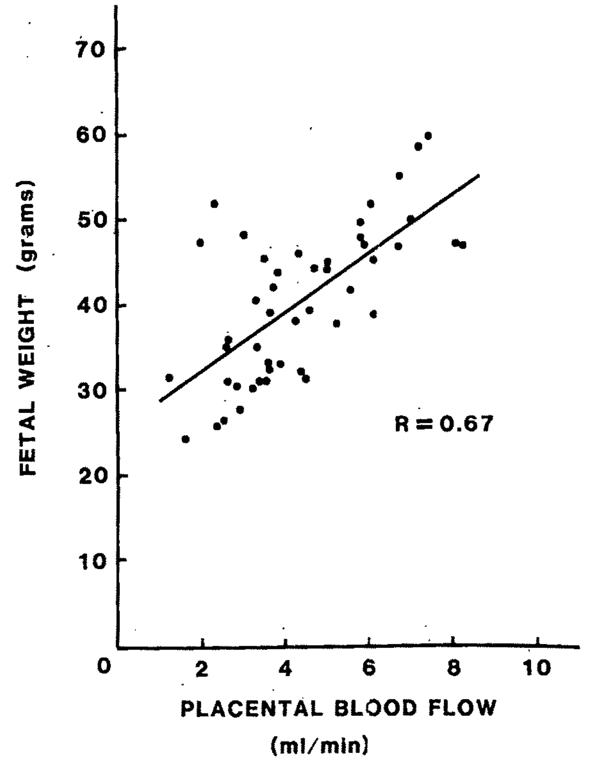

Fig. 2. Relationship of fetal weight and placental blood flow at day 29 of pregnancy in five rabbits, each carrying a large litter of nine to 11 fetuses.

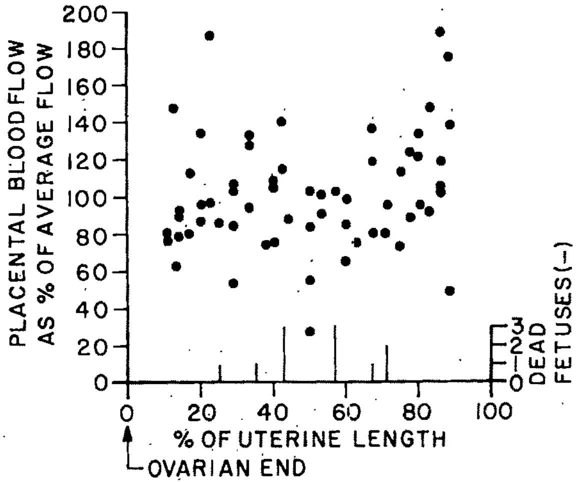

Fig. 3. Relationship of placental blood flow per live fetus to position of placenta in uterine horn. The position of dead fetuses is also indicated.

Comment

Pregnancy requires an increase of uterine blood flow to meet the metabolic demands of the conceptus, and an increase of mammary blood flow in preparation for nursing. Although an increased rate of perfusion of the reproductive organs during pregnancy is a general finding, studies in different species have shown large quantitative differences. Some of these differences may represent methodologic errors and/or differences in the physiologic state of the preparations. In recent years, however, the microsphere technique of blood flow measurement has been applied to conscious, unstressed pregnant sheep, guinea pigs, and rabbits with consistent results within each species among different laboratories. The placental blood flows that were measured in the present study, calculated as flows per gram of placenta, did not differ significantly from blood flows that were measured by two other groups of investigators[2,3] in the pregnant rabbit 1 to 2 days after operation.

A comparison of the rabbit and guinea pig data[1,4] with analogous data in sheep[5] (Table III) demonstrates that, toward the end of pregnancy, blood flow per gram of fetus is approximately 2.5-times higher in sheep. The most likely explanation for this fundamental interspecies difference is that the placenta of the rabbit[6] and the guinea pig[7] is a countercurrent exchanger, whereas the placenta of the sheep is a "venous equilibration exchanger."[8-10] Thus it would seem that in both rabbits and guinea pigs the major physiologic consequence of a highly efficient arrangement of placental blood flows is to allow a relatively small rate of perfusion of the maternal surface of the placental barrier. It is important to note that the perfusion pattern of the villous-hemochorial human placenta is quite different from that of the labyrinthine-hemochorial placenta of the rabbit. This implies that, despite a similarity in the structure of the placental barrier, the perfusion rate of the human placenta may not be comparable to the perfusion rate of the rabbit placenta.

The comparison of pregnant rabbits and guinea pigs is also interesting because the two species have a basic similarity in placental structure and perfusion pattern but differ markedly in the fetal/maternal weight ratio (Table III) and in the maturity of the newborn animal. The guinea pig produces large fetuses that are quite mature at birth and nurse for only a brief period. By contrast, in the rabbit the total fetal mass is relatively small; the offspring are immature at birth and grow rapidly before weaning. Related to these differences is the observation that uterine blood flow is a much larger fraction of cardiac output in the guinea pig than in the rabbit, whereas the opposite is true for mammary blood flow (Table III).

Several investigators have observed that in rabbits placental blood flow varies with the site of implantation. Duncan[11] and Bruce and Abdul-Karim[12] demonstrated a higher placental blood flow in the placenta at the ovarian end of the horn. Katz and Creasy,[2] in a study of nine rabbits with three live fetuses per horn, demonstrated that the middle placenta had a smaller blood flow than the two placentas at the ovarian and vaginal ends. Our observations agree with the latter finding in demonstrating a tendency for the least placental blood flow to occur in the middle of the uterine horn.

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Erythrocyte concentrations and transmembrane fluxes of sodium and potassium and biochemical measurements during the menstrual cycle in normal women

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The erythrocyte concentrations and the transmembrane fluxes of sodium and potassium were investigated in 20 normal women during the two stages of the menstrual cycle. Half of the women were using oral contraceptives and the other half were not. In women with a normal menstrual cycle the erythrocyte sodium concentration and the ouabain-insensitive total potassium efflux were lower in the luteal than in the follicular phase. Intracellular potassium concentration, ouabain-sensitive rubidium 86 uptake and the furosemide-sensitive sodium and potassium efflux did not differ significantly between the two periods of the cycle. No cycle-related variation in sodium or potassium intracellular concentration was observed in women using oral contraceptives. In these women, however, the ouabain-sensitive ^{86}Rb uptake was increased in the second part of the menstrual cycle. In each woman with a normal menstrual cycle the plasma progesterone, renin activity, angiotensin II, plasma aldosterone concentration, and urinary aldosterone excretion increased during the luteal phase. The increment in the plasma renin activity, plasma angiotensin II, and plasma and urinary aldosterone indicate a stimulation of the renin-aldosterone axis in this menstrual period. During the same phase, serum cholesterol was decreased significantly. When the women using oral contraceptives were compared to those not using them, the renin-aldosterone axis was already stimulated during the first part of the cycle; no further stimulation occurred during the second part. (AM J OBSTET GYNECOL 1985;151:687-93.)

Key words: Erythrocyte, sodium, potassium, menstrual cycle, oral contraceptives

In most studies of erythrocyte concentrations and transmembrane fluxes of sodium and potassium, data of men and women are usually combined.[1-5] There is some evidence, however, that sex[6, 7] and possibly hormonal status[8-10] might affect the cellular cationic transport systems.

In the present study the intracellular concentrations of sodium and potassium and their transmembrane fluxes were assayed longitudinally in normal women during the menstrual cycle and in women using a combined oral contraceptive. Changes occurring during the cycle were examined together with concurrent variations in plasma, serum, and urinary levels of several biochemical variables.

Material and methods

Subjects. Twenty nonobese white women were evaluated after their informed consent had been obtained. Ten women had been using a combined low-dose oral contraceptive for at least 6 months, and 10 women had a regular biphasic menstrual cycle. No other drug was taken during the last 4 weeks preceding the study. The subjects were evaluated according to a cross-over design: five women of each group were first investigated during the first half of the menstrual cycle (day 11), and the other five women were first evaluated during the second half (day 22). Thus the latter subjects were investigated in two consecutive menstrual cycles. The luteal phase was ascertained by progesterone assays. On specific questioning three subjects reported the presence of hypertension in their father, mother, or both.

Protocol. The subjects came to the laboratory between 8 and 9:30 AM after an overnight fast. They first completed a questionnaire concerning age, date of last menstruation, past and present diseases, drug intake, and familial background of hypertension. Then body weight and height were measured. The subjects sat in an armchair for 5 minutes before the sitting blood pressure and heart rate were measured. Thereafter the volunteers were allowed a 5-minute relaxation period in the recumbent position, and subsequently the blood pressure and heart rate were determined. Finally, heparinized blood was withdrawn from an antecubital vein.

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Table I. General characteristics of the subjects

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
n	10		10	
Family history of hypertension (n)	2		1	
Age (yr)*	23 ± 2		23 ± 0.3	
Height (cm)*	167 ± 2		168 ± 2	
Weight (kg)*	59.7 ± 2.3	60.3 ± 2.1	60.8 ± 2.2	60.9 ± 2
Blood pressure (mm Hg)*				
Recumbent				
Systolic	101 ± 2	93 ± 3	96 ± 9	106 ± 3
Diastolic	62 ± 3	61 ± 2	64 ± 1	56 ± 6
Sitting				
Systolic	110 ± 2	106 ± 3	113 ± 4	113 ± 3
Diastolic	68 ± 3	67 ± 2	70 ± 1	68 ± 3
Heart rate (bpm)*				
Recumbent	71 ± 2	73 ± 2	74 ± 4	71 ± 4
Sitting	80 ± 2	82 ± 2	80 ± 4	71 ± 8

*Mean ± SE.

Table II. Erythrocyte concentration of sodium and potassium during the menstrual cycle*

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Sodium intercellular concentration (mmol/L of cells)	7.24 ± 0.44	6.62 ± 0.29†	6.85 ± 0.27	7.08 ± 0.33
Potassium intercellular concentration (mmol/L of cells)	84.7 ± 2.2	79.8 ± 1.1	84.8 ± 2.7	84.5 ± 2.5

*Mean ± SEM.

†$p < 0.05$ when compared to day 11 by means of paired *t* test.

Intraerythrocyte concentration of sodium and potassium and the activity of the sodium-potassium-adenosinetriphosphatase, estimated from the ouabain-sensitive uptake of rubidium 86 by the red blood cells, were assayed as reported elsewhere.[11] The sodium-potassium cotransport activity was assessed by the furosemide-sensitive sodium and potassium efflux in sodium-loaded and potassium-depleted red blood cells by subtracting the efflux rate in the presence of furosemide from the one in the absence of furosemide.[4]

The sodium-lithium countertransport or the sodium-stimulated lithium efflux was calculated by subtracting the lithium efflux assayed in lithium-loaded red blood cells in the sodium-free medium from that in the sodium-enriched medium.[3]

Plasma renin activity[12] and substrate,[13] plasma angiotensin II,[14] plasma aldosterone concentration,[15] progesterone,[16] serum electrolytes, cholesterol, and creatinine values were determined. Twenty-four hour urine collections were analyzed for sodium, potassium, calcium, magnesium, phosphate, creatinine, urinary aldosterone,[17] kallikrein,[18] and prostaglandins.[19]

Statistical analysis. Data are represented by the mean ± SEM except for plasma renin activity, plasma angiotensin II, plasma aldosterone concentration, and urinary aldosterone excretion, for which the geometric mean and range are given, since log distributions were closer to a gaussian curve. Paired and unpaired Student's *t* tests were applied where appropriate. Regression analyses were also performed.

Results

General characteristics of the subjects. The women with a natural cycle and the women using oral contraceptives were comparable in age, height, weight, blood pressure, and heart rate. In both groups, weight, blood pressure, and heart rate were similar during the first (day 11) and the second (day 22) half of the menstrual cycle (Table I).

Intracellular sodium and potassium concentration. In women with a normal menstrual cycle the erythrocyte sodium concentration was lower ($p < 0.05$) during the luteal than during the follicular phase (Table II). The red blood cell potassium intracellular concentration was not significantly different in either phase of the cycle. In another group of women with a normal

Table III. Red blood cell ^{86}Rb uptake during the menstrual cycle

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Total	3.43 ± 0.18	3.24 ± 0.14	3.34 ± 0.17	3.51 ± 0.15
Ouabain-resistant	0.79 ± 0.08	0.75 ± 0.07	0.88 ± 0.11	0.90 ± 0.10
Ouabain-sensitive	2.64 ± 0.13	2.49 ± 0.14	2.46 ± 0.13	2.61 ± 0.33*

Mean ± SEM.
*$p < 0.05$ when compared to day 11 by means of paired *t* test.

Table IV. Ouabain-insensitive sodium-potassium effluxes during the menstrual cycle

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Sodium effluxes (μmol/L of cells/hr)				
Total	945 ± 66	885 ± 58	1269 ± 153	1245 ± 182
Furosemide-resistant	657 ± 45	615 ± 41	857 ± 99	831 ± 126
Furosemide-sensitive	288 ± 45	270 ± 41	412 ± 65	414 ± 76
Potassium effluxes (μmol/L of cells/hr)				
Total	994 ± 40	939 ± 41*	1042 ± 66	1024 ± 66
Furosemide-resistant	565 ± 29	565 ± 18	524 ± 24	508 ± 23
Furosemide-sensitive	429 ± 47	374 ± 42	518 ± 56	516 ± 66

Mean ± SEM.
*$p < 0.05$ when compared to day 11 by means of paired *t* test.

Table V. Lithium effluxes during the menstrual cycle

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Lithium effluxes (μmol/L of cells/hr)				
In sodium-enriched medium	501 ± 33	483 ± 27	587 ± 62	609 ± 62
In sodium-free medium	143 ± 9	124 ± 8*	176 ± 59	184 ± 20†
Countertransport	358 ± 30	359 ± 28	411 ± 49	425 ± 51

Mean ± SEM.
*$p < 0.05$ when compared to day 11 by means of paired *t* test.
†$p < 0.05$ when compared to the luteal phase in nonusers.

menstrual cycle who were studied in similar conditions, sodium intracellular concentration was also found to be lower ($p < 0.05$) in the luteal phase (6.7 ± 0.28 mmol/L of cells, n = 12) than during the follicular phase (7.8 ± 0.42 mmol/L of cells, n = 9).

In the women using oral contraceptives neither sodium nor potassium intracellular concentration were significantly different between day 22 and day 11 of the cycle. Neither during the first (day 11) nor during the second half (day 22) of the cycle were the sodium and potassium intracellular concentrations significantly different between the women with a normal menstrual cycle and those using oral contraceptives.

Sodium-potassium-adenosinetriphosphatase activity. In the total group of women with a natural menstrual cycle, the ouabain-resistant ^{86}Rb uptake and the activity of the sodium-potassium-pump assessed by the ouabain-sensitive ^{86}Rb uptake did not differ significantly between the follicular and the luteal phase of the menstrual cycle (Table III). In users of oral contraceptives, on the contrary, the ouabain-sensitive ^{86}Rb uptake was higher ($p < 0.05$) in the second than in the first part of the cycle; the total ^{86}Rb uptake and the ouabain-resistant fraction did not change. The comparison between women using oral contraceptives and women with a normal cycle did not reveal any significant differences.

Sodium-potassium cotransport activity. No cycle-induced difference in total, furosemide-resistant and furosemide-sensitive sodium effluxes was found in

Table VI. Plasma and serum measurements during the menstrual cycle

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Serum				
Sodium (mEq/L)	140.6 ± 0.3	141.4 ± 0.7	140.0 ± 0.5	140.0 ± 0.7
Potassium (mEq/L)	4.02 ± 0.1	4.8 ± 0.18*	4.94 ± 0.34†	4.75 ± 0.42
Total calcium (mg%)	9.28 ± 0.12	9.3 ± 0.13	9.04 ± 0.12	9.06 ± 0.13
Magnesium (mg%)	1.97 ± 0.02	1.95 ± 0.04	1.97 ± 0.04	1.99 ± 0.04
Phosphate (mg%)	3.36 ± 0.12	4.91 ± 0.86	2.91 ± 0.23	3.52 ± 0.93
Creatinine (mg%)	0.88 ± 0.05	1.01 ± 0.07	0.94 ± 0.04	0.92 ± 0.05
Cholesterol (mg%)	193 ± 9.8	179 ± 10.4‡	181 ± 8.5	187 ± 7.6
Plasma				
Plasma renin substrate (μg/ml)	1.24 ± 0.08	1.25 ± 0.06	3.6 ± 0.25§	4.37 ± 0.19§*
Plasma renin activity (ng/ml/hour)‖	0.44 (0.19-0.89)	0.70* (0.28-1.29)	0.70† (0.28-1.17)	0.52‡ (0.24-1.10)
Plasma angiotensin II (pg/ml)‖	52 (17.5-108.1)	100‡ (50.5-225.8)	100† (59.1-162.9)	88.9 (50.7-208.1)
Plasma aldosterone concentration (ng%)‖	11.5 (5.8-22.5)	16‡ (9.4-26.6)	15.2 (8.0-24.6)	15 (11.4-24.1)
Progesterone (ng/ml)	1.06 ± 0.2	7.63 ± 2.18‡	0.67 ± 0.06¶	0.69 ± 0.04¶

Mean ± SEM.
*$p < 0.01$ when compared to day 11 by means of paired *t* test.
†$p < 0.05$ when compared to similar day in nonusers.
‡$p < 0.05$ when compared to day 11 by means of paired *t* test.
§$p < 0.001$ when compared to similar day in nonusers.
‖Geometric mean (and range).
¶$p < 0.01$ when compared to similar day in nonusers.
#$p < 0.001$ when compared to day 11 by means of *t* test.

women with natural cycles (Table IV). There was no significant difference between the two phases in the furosemide-resistant and the furosemide-sensitive potassium efflux. The total potassium efflux, however, was lower ($p < 0.05$) during the luteal than during the follicular phase of the cycle.

In women using oral contraceptives the total, furosemide-resistant and furosemide-sensitive sodium and potassium effluxes did not change during the cycle.

No significant differences were observed between the women using oral contraceptives and the women with a natural menstrual cycle.

Sodium-lithium countertransport activity. The lithium efflux in sodium-free ($p < 0.05$) medium was lower during the luteal than during the follicular phase in women with natural menstrual cycles (Table V). The lithium efflux in sodium-enriched medium and the calculated sodium-lithium countertransport were unchanged during the menstrual cycle.

There was no cycle-related change in lithium efflux in the women using oral contraceptives.

In the second part of the cycle the lithium efflux in sodium-free medium was higher ($p < 0.05$) in the women using oral contraceptives than in women with a natural cycle.

Other biochemical variables. In normal women a significant increase in serum potassium ($p < 0.01$), plasma renin activity ($p < 0.01$), plasma angiotensin II ($p < 0.05$), plasma aldosterone concentration ($p < 0.05$), and plasma progesterone ($p < 0.05$) occurred during the luteal phase, whereas serum cholesterol was decreased ($p < 0.05$) (Table VI). The increase in plasma progesterone during the luteal phase occurred in each single subject. Also the 24-hour urinary excretion of aldosterone ($p < 0.05$), kallikrein ($p < 0.01$), and 6-K-i prostaglandin $F_{1\alpha}$ increased significantly in the luteal phase (Table VII).

In the women using oral contraceptives plasma renin activity decreased ($p < 0.05$) whereas plasma renin substrate increased ($p < 0.01$) in the second part of the cycle. The other plasma, serum, and urinary variables did not change significantly.

In the first part of the menstrual cycle, serum potassium ($p < 0.05$), plasma renin substrate ($p < 0.001$), plasma renin activity ($p < 0.05$), plasma angiotensin II ($p < 0.05$), and urinary aldosterone excretion ($p < 0.05$) were higher in women using oral contraceptives than in women with a natural cycle. In the second part of the cycle both groups were comparable for most of the biochemical variables, but plasma renin substrate was still higher ($p < 0.001$) and plasma progesterone remained lower ($p < 0.01$) in women using oral contraceptives.

Regression analysis. Single regression analysis was

Table VII. Urinary measurements during the menstrual cycle

	Normal menstrual cycle		*Use of oral contraceptives*	
	Follicular phase (day 11)	*Luteal phase (day 22)*	*First half (day 11)*	*Second half (day 22)*
Volume (ml/24 hr)	1135 ± 163	1325 ± 146	1183 ± 121	1260 ± 154
Sodium (mmol/24 hr)	143 ± 16	164 ± 20	120 ± 11	125 ± 9
Potassium (mmol/24 hr)	60 ± 6	69 ± 4	58 ± 6	52 ± 4*
Calcium (mg/24 hr)	160 ± 36	164 ± 21	135 ± 24	120 ± 18
Magnesium (mg/24 hr)	137 ± 32	99 ± 7	91 ± 14	147 ± 48
Creatinine (gm/24 hr)	1.38 ± 0.09	1.33 ± 0.07	1.38 ± 0.1	1.45 ± 0.1
Kallikrein (U/24 hr)	0.755 ± 0.138	0.937 ± 0.140†	0.716 ± 0.105	0.730 ± 0.155
Aldosterone (μg/24 hr)‡	2.3 (1.4-5.9)	3.8§ (2.0-8.5)	5.2‖ (1.8-12.3)	4.8 (2.6-12.6)
i prostaglandin E_2 (ng/24 hr)	280 ± 44	283 ± 30	664 ± 215	501 ± 142
i prostaglandin $F_{2\alpha}$ (ng/24 hr)	1025 ± 158	2042 ± 137	2560 ± 230	2272 ± 135
6-K-i prostaglandin $F_{1\alpha}$ (ng/24 hr)	1002 ± 58	1172 ± 61§	1292 ± 74*	1127 ± 82

Mean ± SEM.

*$p < 0.01$ when compared to similar day in nonusers by means of unpaired t test.

†$p < 0.01$ when compared to day 11 by means of paired t test.

‡Geometric mean (and range).

§$p < 0.05$ when compared to day 11 by means of paired t test.

‖$p < 0.05$ when compared to similar day in nonusers by means of unpaired t test.

performed separately in the oral contraceptive users and in the women with a normal cycle with sodium and potassium intracellular concentrations as dependent variables. For both phases of the menstrual cycle, no relationship was found between either sodium or potassium intracellular concentration and age, weight, blood pressure, or plasma, serum, and urinary variables.

In women with a normal menstrual cycle the change in sodium intracellular concentration between the follicular and the luteal phase of the cycle was correlated with concurrent variation in potassium intracellular concentration ($r = 0.89$, $p < 0.001$). The change in potassium intracellular concentration was also correlated with that in serum potassium ($r = -0.68$, $p < 0.001$). No other significant correlations were found between changes of either sodium or potassium intracellular concentration and concurrent variations in measurements of plasma, serum, and urinary variables or fluxes.

In women using oral contraceptives, no significant correlation was found between the change in sodium or potassium intracellular concentration and concomitant changes in other variables.

Comment

The purpose of the present study was to investigate (1) the change in sodium and potassium concentrations and fluxes in the erythrocytes of normal women during the menstrual cycle and (2) the effect of the oral contraceptive pill. Possible fluctuations could be of methodologic and physiologic relevance. Indeed, if such variations were observed, future studies in women should take into account the period in which the subjects were investigated. Furthermore, the mechanism of such variation could be of physiologic importance.

Several other variables were determined at the same time in order to detect possible relationship between their changes during the cycle and those occurring in the fluxes.

Intracellular sodium concentration. The main finding in the present study was the lower red blood cell concentration of sodium in the luteal than in the follicular phase of the menstrual cycle. The present observation in 10 women investigated longitudinally in both parts of the menstrual cycle confirms our results obtained from a cross-sectional study in which 21 normal women were studied under similar conditions either in the follicular ($n = 9$) or in the luteal phase ($n = 12$). These studies, however, disagree with the statement of Love and Burch[20] that the concentration of sodium in the red blood cells of women did not change during the menstrual cycle. The actual figures, however, were not given in their study.

It remains unclear which alteration in the transmembrane transport system caused the lower sodium intracellular concentration in the luteal phase, since neither the sodium pump activity estimated from the ouabain-sensitive ^{86}Rb uptake nor the outward cotransport of sodium, estimated from the difference in the total and the furosemide-resistant sodium efflux, was enhanced. Instead, both transport systems tended to decrease in the luteal phase. However, the passive permeability to sodium was not determined in the present study. Krzesinski and Rorive[21] reported a higher sodium cotransport activity in the luteal as compared to the follicular phase. Unfortunately, the sodium intracellular concentration was not simultaneously determined in their study, so it is uncertain whether the higher cotransport resulted in a lower sodium intracellular concentration.

The use of oral contraceptives suppressed the observed difference in sodium intracellular concentration between the two stages of the cycle. It is tempting to imagine that oral contraceptives alter the normal hormonal homeostasis that, in nonusers, could be responsible for the sodium intracellular concentration lowering in the luteal phase. The constant level of plasma progesterone, aldosterone concentration, and urinary aldosterone excretion with unchanged sodium intracellular concentration in the two parts of the menstrual cycle in the users of oral contraceptives contrasts with the variations in both sodium intracellular concentration, progesterone, and plasma and urinary aldosterone observed in women with natural menstrual cycles.

Intracellular potassium. The erythrocyte potassium concentration did not change significantly during the natural menstrual cycle, confirming the finding of Love and Burch.[20] Beilin et al.,[22] on the contrary, observed a day-to-day progressive decrease in red blood cell potassium from day 3 to day 26 of the cycle. In the latter study, however, no mention was made of the statistical significance of the decrease.

As in women with natural cycles, potassium intracellular concentration did not change in women using oral contraceptives, despite an increment in the sodium-potassium-adenosinetriphosphatase activity during the second half of the menstrual cycle (Table III). Since the potassium outward cotransport activity remained unchanged, the increment in ouabain-sensitive ^{86}Rb uptake (which is an expression of the potassium uptake by the cell) must have been counterbalanced by an increased passive outflux of potassium.

Biochemical changes during the menstrual cycle in normal women. The observed rise in plasma progesterone concentration during the luteal phase in each woman with a natural menstrual cycle was expected and it confirmed that the subjects were indeed studied during the follicular and the luteal phases of the cycle (Table VI). The increased plasma renin activity, plasma angiotensin II, plasma aldosterone concentration, and urinary aldosterone excretion during the luteal phase indicated a stimulation of the renin aldosterone axis.[23-25] It is thought to be related to the antialdosterone effect of progesterone[25] which may also be responsible for the increment in serum potassium during the luteal phase. The reasons for increase in urinary kallikrein and 6-K-i prostaglandin $F_{1\alpha}$ during the luteal phase are not clear; their increment was not related to the stimulation of the renin-aldosterone axis.

Serum cholesterol decreased in the luteal phase when sodium intracellular concentration also decreased. Studies showing either a cyclic rise[26, 27] or a cyclic decrease[28, 29] in total serum cholesterol, with the highest or the lowest concentrations at the moment of ovulation or in the luteal phase, have been reported, with other studies[30] describing no change at all. Thus it is uncertain whether the association of a lower serum cholesterol with a decreased sodium intracellular concentration during the luteal phase in the present study could be causative.

Biochemical changes induced by oral contraceptives. Compared to women with natural menstrual cycles, users of oral contraceptives had higher plasma renin substrate, renin activity, angiotensin II, plasma aldosterone concentration, and urinary aldosterone excretion during the first part of the menstrual cycle, which confirmed previous reports.[23, 25] During the second part of the cycle, however, no further stimulation of the renin-aldosterone system occurred in users of oral contraceptives, in contrast with women with normal menstrual cycles. During the second half of the cycle the differences in renin, angiotensin II, and plasma and urinary aldosterone observed during the first half between users of oral contraceptives and normal women thus disappeared. The higher serum potassium observed in women using oral contraceptives during the first part of the cycle could be attributed to the progesterone content of the contraceptive used.

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The effect of medroxyprogesterone acetate on premature labor in the sheep

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The ability of medroxyprogesterone acetate to delay premature delivery was investigated in nine chronically catheterized pregnant sheep (125 to 130 days' gestation). Catheters were placed for measurement of intrauterine pressure and uterine vein concentrations of progesterone, 17β-estradiol, and 15-keto-13,14-dihydroxyprostaglandin $F_{2\alpha}$. Premature parturition was induced in all animals by infusion of dexamethasone (1 mg/24 hours) to the fetus. Three ewes served as controls, three ewes received oral medroxyprogesterone acetate (5 mg/kg/day) 48 hours prior to the start of and during dexamethasone (early medroxyprogesterone acetate), and the remaining three animals received oral medroxyprogesterone acetate 12 hours after the onset of dexamethasone infusion (late medroxyprogesterone acetate). The lengths of time from dexamethasone until the onset of labor and from dexamethasone until delivery were compared, and were significantly longer for each group compared to control times. Of all endocrine events analyzed, the fall in plasma progesterone was the most consistent in all groups, showing that animals with rapid falls in progesterone levels (control group) tended to deliver earlier. These results demonstrate that medroxyprogesterone acetate can delay premature parturition in the sheep. Also medroxyprogesterone acetate appears to delay the endocrinologic events that normally occur at this time. (AM J OBSTET GYNECOL 1985;151:694-700.)

Key words: Medroxyprogesterone acetate, premature labor, dexamethasone

Observations made by Liggins et al.[1] demonstrated that, in sheep, the fetal pituitary-adrenal axis during late pregnancy was of importance in the mechanism of parturition. The interplay of these organs results in an increase of the secretion rate of cortisol by the fetal adrenal gland and of the concentration of cortisol in the fetal circulation. We now recognize that this rise of plasma cortisol represents an early link in a chain of endocrine events leading to parturition. The rise in fetal cortisol is followed by a decrease in the concentration of progesterone in maternal plasma. This fall in placental progesterone output is the result of an increase of 17α-hydroxylase activity in the placenta, which causes progesterone to be metabolized to a less active product, 17,20-dihydroxypregn-4ene-3one. Under the influence of fetal cortisol, the increased availability of 17-hydroxylated C21 steroids in the placenta contributes to the sharp rise in unconjugated estrogens in maternal blood.[2] These changes in maternal steroid concentrations produce several different physiologic effects, which may result in the onset of labor in the sheep. They act on decidua and fetal membranes to increase prostaglandin production,[3] on maternal pituitary to facilitate oxytocin release,[4] and on the myometrium to augment responsiveness to oxytocic agents[5] either by influencing appropriate receptor concentrations[6] or by provoking ultrastructural changes that enhance electrical conductivity.[7]

Although the effects of progesterone in maintaining uterine quiescence have been well established in some species,[8] the use of progesterone in preventing premature labor is controversial. Bengtsson and Schofield,[9] in 1963, found that pregnancy was prolonged in sheep when progesterone was administered daily.

Medroxyprogesterone acetate has gained widespread acceptance as a useful, effective progestational agent with prolonged duration of action. The aim of this study was to investigate whether medroxyprogesterone acetate could delay labor. To this end we have measured daily concentrations of progesterone, estradiol, and a metabolite of prostaglandin $F_{2\alpha}$ and medroxyprogesterone acetate as well as intrauterine pressures before and during the induction of premature labor by dexamethasone infusion to the fetus in control and medroxyprogesterone-treated groups. The findings were similar to those of Liggins et al.,[1] who used

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Table I. Characteristics of steroid radioimmunoassays

	Progesterone	*Estradiol*	*Prostaglandin $F_{2\alpha}$ metabolite*	*Medroxyprogesterone acetate*
Range of linearity of standard curves (pg)	20-2000	6.25-500	20-1000	20-2000
Slope of standard curves (mean ± SD)	0.798 ± 0.067 (n = 36)	0.969 ± 0.069 (n = 16)	0.851 ± 0.054 (n = 14)	0.922 ± 0.045 (n = 16)
Slope of sheep plasma	—*	—*	0.979 ± 0.176† (n = 5‡)	1.119 ± 0.042† (n = 5)
Mass recovery	—*	—*	y = 1.01 ± 0.29	y = 1.07 ± 1.22
Correlation coefficient			0.991	0.998
Recovery of internal standard (%)	85-95	70-80	65-75	90-95
Sensitivity (pg/assay)	20	6.25	20	20
Coefficients of Variations				
Intra-assay	11.38 (n = 5)	8.4 (n = 5)	4.7 (n = 6)	6.75 (n = 6)
Interassay	17.4 (n = 36)	29.77 (n = 36)	14.1 (n = 18)	9.14 (n = 14)

*Previously validated, reference 11.

†Not different from slope of standard curve.

‡Number of samples.

progesterone in oil; when medroxyprogesterone acetate was administered, not only were the fall in progesterone and the rise of estradiol delayed, but also the onset of labor was postponed.

Methods and material

Nine pregnant ewes of mixed breed between 115 and 125 days of pregnancy, whose gestational age was determined by x-ray evaluation of fetal bone maturation and breeding date, were starved for a period of 48 hours before operation. A spinal anesthetic 50% sodium pentobarbital for induction of anesthesia were given as previously described.[10] A polyvinyl catheter (PV_3, Bolab) was implanted into a fetal lateral tarsal vein for infusion of dexamethasone.[11] A polyvinyl catheter (PV10, Bolab) was placed free in the amniotic cavity to record intrauterine pressures. Another polyvinyl catheter (PV_5, Bolab) was placed in the maternal uterine vein for samplings. Ampicillin, 500 mg, was injected into the amniotic cavity. Penicillin (600,000 U) and streptomycin (0.5 gm) were administered on the day of operation and on the first three postoperative days.

After operation, the animals were fed water and food as desired, and they remained in the laboratory stalls throughout the period of experimentation. After a 7-day recovery period, dexamethasone (1 mg/24 hours) was infused into the fetus until delivery. Three ewes received 5 mg/kg of medroxyprogesterone acetate by mouth daily at 8:00 AM, starting 48 hours prior to and continuing throughout the infusion of dexamethasone (early medroxyprogesterone acetate groups). Three more ewes received 5 mg/kg of medroxyprogesterone acetate by mouth 12 hours after dexamethasone was started and every 24 hours thereafter (late medroxyprogesterone acetate group). However, no sheep received medroxyprogesterone acetate treatment longer than 4 days after initiation of dexamethasone infusion. The remaining three received no medroxyprogesterone acetate and served as controls.

Physiologic measurements. Measurements of progesterone, estradiol, and prostaglandin $F_{2\alpha}$ metabolite and medroxyprogesterone acetate were made daily for 4 to 6 days prior to dexamethasone infusion, every 2 hours for the first 8 hours of the infusion, and every 4 hours thereafter. Intrauterine pressure was recorded continuously on the day before and during the period of dexamethasone infusion by previously described methods.[10] The onset of frequent uterine contractions (every 4 to 5 minutes) of 5 to 10 mm Hg intensity marked the onset of labor.

Blood sample collection. A total of 8 ml of blood was drawn on ice in heparinized tubes from the uterine vein at each sample. After collections, plasma was separated by centrifugation at 4° C at 3000 × g for 10 minutes. Plasma samples were stored at −20° C until hormonal analysis was performed. Fetal blood pH and maternal hematocrit were measured daily to determine fetal and maternal well-being.

Radioimmunoassay

Progesterone assay. Sheep plasma (0.2 ml) to which 1000 cpm of tritium-labeled progesterone (New England Nuclear, Boston, Massachusetts) had been added to calculate procedure losses was extracted with 6 ml of petroleum ether and assayed as previously described.[11]

Estradiol assay. Sheep plasma (1.0 ml) was extracted with 6 ml of anhydrous ether, air-dried, purified on a Sephadex LH-20 column, and assayed as previously described.[11] The estradiol antiserum (029) used in this assay was found to be cospecific for estrone and 17β-

Table II. Gestational ages, fetal weights, hours of dexamethasone infusion, and duration of labor in sheep treated with medroxyprogesterone acetate before and after dexamethasone infusion

	Group 1: Control	*Group 2: Early medroxy progesterone acetate*	*Group 3: Late medroxy progesterone acetate*
Operation	121 + 2	123 ± 1	118 ± 1
Dexamethasone infusion	130 ± 2	130 ± 1	125 ± 1
Delivery	132 ± 2	134 ± 1	128 ± 1
Fetal weight (kg) (mean ± SE)	3 ± 0.5	3.2 ± 0.3	2.5 ± 0.3
Hours of dexamethasone infusion to onset of labor (mean ± SE)	38 ± 2	94 ± 17*	57 ± 2*
Hours of labor (mean + SE)	7 ± 0.5	12 ± 2†	13 ± 1†
Hours of dexamethasone infusion (mean ± SE)	45 ± 2	100 ± 15‡	70 ± 2‡

*Statistically significantly different from control (one-way analysis of variance: $p<0.05$).
†Statistically significantly different from control (one-way analysis of variance: $p<0.05$).
‡Statistically significantly different from control (one-way analysis of variance: $p<0.05$).

estradiol and also to cross react with 17β-estradiol (25%) and estriol (10%). Since the Sephadex LH-20 column cannot separate 17β-estradiol and 17α-estradiol, the results are reported as total estradiol and expressed as 17β-estradiol equivalents.

Prostaglandin $F_{2\alpha}$ metabolite (13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$) assay. The technique used was modified from the method developed by Haning et al.[12] Plasma (0.5 ml) and 100 cpm of tritium-labeled prostaglandin $F_{2\alpha}$ metabolite (Amersham, Chicago, Illinois) were mixed uniformly and acidified with 0.1 ml of 0.1N hydrochloric acid. Petroleum ether (3 ml) was added to this acidified plasma to wash away the neutral lipid. Prostaglandins were then extracted by the addition of ethyl acetate (4 ml). The extract was transferred to conical tubes, air-dried, and subjected to silicic acid column chromatography.

The elution solvents for column chromatography were prepared fresh daily. The ratio of the volumes of toluene, ethyl acetate, and methanol were as follows: solvent 1, 85:15:0; solvent 2, 60:40:0.5; solvent 3, 60:40:5, solvent 4, 60:40:20.

Column chromatography was performed on a Brock mini column (15 by 1 cm) packed with silicic acid slurry. One gram of silicic acid (Unisil, 100 to 200 mesh, Clarkson Chem Co., Inc., Williamport, Pennsylvania) was suspended in 6 ml of solvent 4 and poured into the column previously washed with 10 ml of solvent 1. The extracted sample was dissolved with 0.4 ml solvent 4 mixed with 0.4 ml of solvent 1 and applied to the column. The extraction tube was rinsed with 0.3 ml of solvent 1 to ensure more complete transfer of the sample. The column was washed sequentially with 10 ml of solvent 1, 5 ml of solvent 2, 2 ml of solvent 3, and 8 ml of solvent 3. The first three eluates were discarded. The prostaglandin $F_{2\alpha}$ metabolite was eluted in the final 8 ml fraction. Radioimmunoassay was performed in this purified fraction. Samples were mixed with 0.1 ml of antiserum and 500 cpm of tritium-labeled prostaglandin $F_{2\alpha}$ metabolite (Amersham, Chicago, Illinois) and incubated at 4° C for 3 hours.

The prostaglandin $F_{2\alpha}$ metabolite antiserum (560-JCC-140D, The Upjohn Co., Kalamazoo, Michigan) did cross react with the prostaglandins as follows: prostaglandin $F_{2\alpha}$ metabolite, 100%; 15-keto-prostaglandin $F_{2\alpha}$, 20%; and the C_{16} metabolite of prostaglandin $F_{2\alpha}$, 13,14-dehydro-prostaglandin $F_{2\alpha}$, and prostaglandin $F_{2\alpha}$, <1%. A cochromatography technique was applied to confirm the specificity of this assay. A total of 1.0 ml of sheep plasma was chromatographed with 1200 cpm of tritium-labeled prostaglandin $F_{2\alpha}$ metabolite. The effluent was collected in 1.0 ml fractions. Aliquots were assayed for radioactivity and for immunoactivity. The elution patterns for tritium-labeled prostaglandin $F_{2\alpha}$ metabolite and endogenous unlabeled prostaglandin $F_{2\alpha}$ metabolite were identical.

Medroxyprogesterone acetate assay. The medroxyprogesterone acetate assay is a modification of the technique of Cornette et al.[13] Phosphate buffer (0.01 mol/L of phosphate buffer, pH 7.4) was used instead of Tris buffer. Also 0.25% dextran-coated charcoal suspension was substituted for the second antibody used by Cornette et al. to separate bound from unbound tracer.

Plasma (0.2 ml) with 1000 cpm of tritium-labeled medroxyprogesterone acetate (New England Nuclear, specific activity of 60 Ci/mmol) was extracted with 5 ml of petroleum ether. The sample was centrifuged and the aqueous phase was frozen in dry ice. The petroleum ether phase was decanted into a vial and air-dried. The

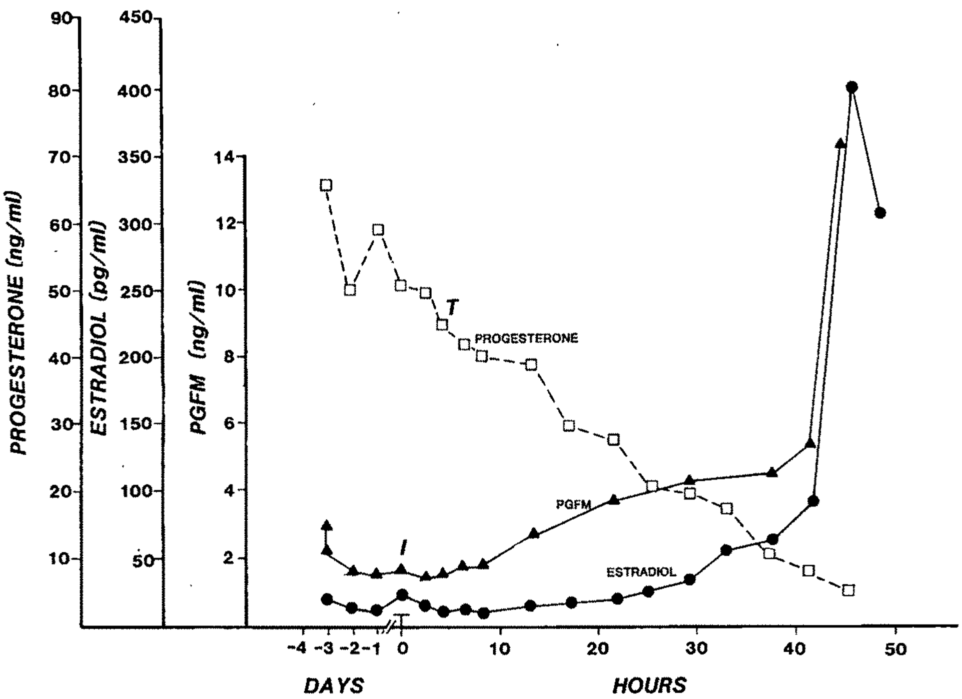

Fig. 1. Mean serial hormonal concentration in maternal plasma in three control sheep with dexamethasone-induced labor. *PGFM* = 15-Keto-13,14-dihydroxyprostaglandin $F_{2\alpha}$. *I* = Start of dexamethasone infusion. *T* = Onset of progesterone drop.

dried extract was reconstituted with 1 ml of absolute ethanol and aliquots of 0.2 ml and 0.4 ml were used for radioimmunoassay.

The medroxyprogesterone acetate (rabbit anti-Provera 15784-ICC-62, The Upjohn Co.) did not cross react with progesterone similarly the progesterone antiserum (P294) used for the progesterone assay did not cross react with MPA.

All the solvents used in these assays were nanograde (Mallinckrodt Chemical, Inc., St. Louis, Missouri). The characteristics of each assay are outlined in Table I. The dilution curves of sheep plasma were parallel to the standard curves so that the slopes of the plasma dilution curves were not significantly different from those obtained with standards (Table I). Mass recovery experiments were performed to check the accuracy of these assays. All these assays showed small variance for both intra-assay and interassay (Table I).

Results

The gestational ages of the sheep at operation, dexamethasone treatment, and delivery are shown in Table II.

Onset of labor. The time from beginning of dexamethasone infusion to the onset of labor was prolonged in the animals receiving medroxyprogesterone acetate. Those receiving medroxyprogesterone acetate before dexamethasone administration took 94 ± 17 (mean ± SE) hours before labor started. In those animals who received medroxyprogesterone acetate after dexamethasone was started, 57 ± 2 hours elapsed before labor began as compared to a control of 38 ± 2 hours. All these results were statistically significant ($p < 0.05$) for both groups compared to control values (Table II).

Length of labor. The duration of labor was the same in both medroxyprogesterone acetate–treated groups (13 ± 1 and 12 ± 2 hours) but significantly prolonged when compared to control (7 ± 0.5 hours, $p < 0.05$). Thus both medroxyprogesterone acetate–treated groups received longer dexamethasone treatments until delivery compared to the control group (Table II).

Hormonal results. Plasma hormonal concentrations varied between individual sheep and among groups (Figs. 1 to 3). In the early medroxyprogesterone acetate group as in the control group, a drop of progesterone or an increase of estradiol and prostaglandin $F_{2\alpha}$ metabolite precedes the onset of labor but these events are significantly delayed (Fig. 2).

In the late medroxyprogesterone acetate group as in the previous group, the patterns of progesterone withdrawal and rises in estradiol and prostaglandin $F_{2\alpha}$ metabolite were similar but the events were less prolonged than in the early medroxyprogesterone acetate group (Fig. 3).

Of all the animals only one sheep in the early medroxyprogesterone acetate group did not go into labor within 24 hours of the last medroxyprogesterone acetate dose. In this animal plasma medroxyprogesterone

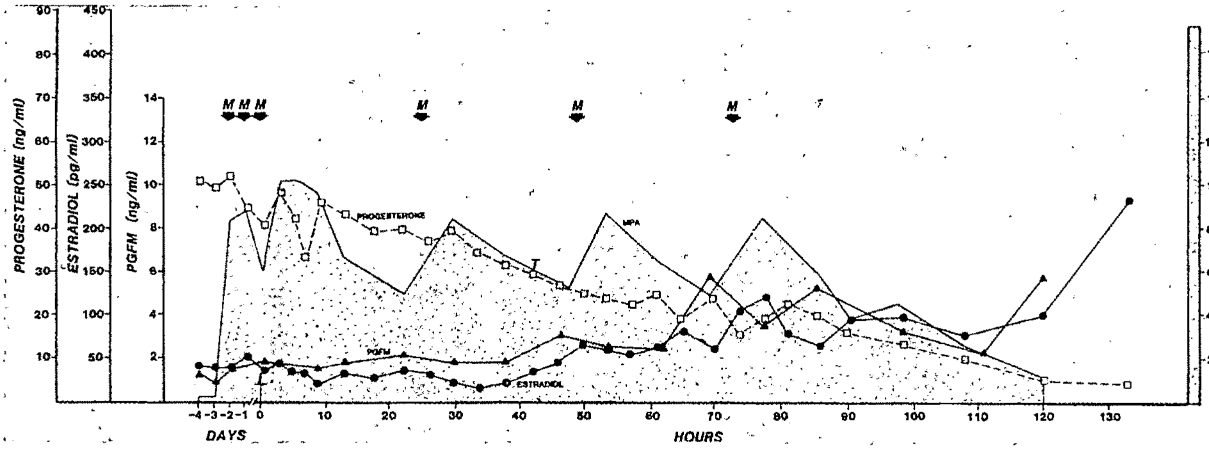

Fig. 2. Mean serial hormonal concentration in maternal plasma in three sheep treated with medroxyprogesterone acetate prior to dexamethasone infusion (early medroxyprogesterone acetate). *M* = Medroxyprogesterone acetate administration. *MPA* = Medroxyprogesterone acetate concentration. Other abbreviations as in Fig. 1.

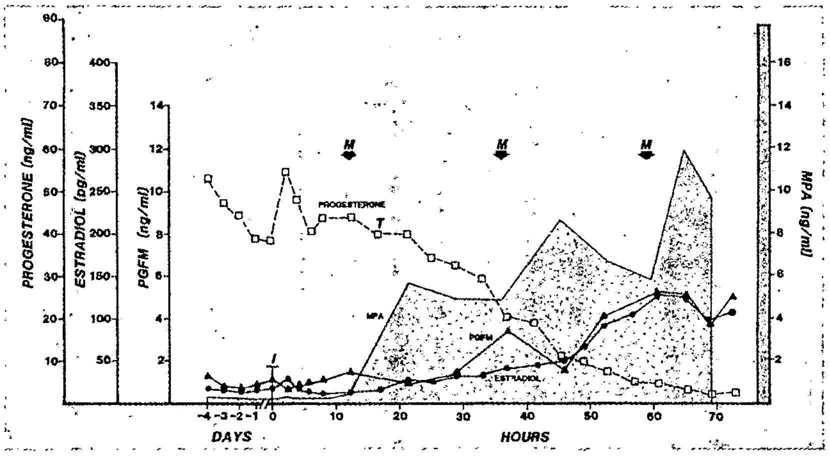

Fig. 3. Mean serial hormonal concentration in maternal plasma in three sheep treated with medroxyprogesterone acetate after dexamethasone infusion (late medroxyprogesterone acetate). Abbreviations as in Figs. 1 and 2.

acetate could be detected, although in small amounts, more than 32 hours after administration.

Of all endocrine events analyzed, the drop in plasma progesterone was the most consistent in all groups. Plasma progesterone was considered to have decreased when its concentration dropped below the 95% confidence limits of the mean of preceding determinations (*T* in Figs. 1, 2, and 3). The time between initiation of dexamethasone infusion and the progesterone drop so defined (*T*) was called phase 1, while the time following such a drop until delivery was called phase 2.

When the slopes of the increases of estradiol and prostaglandin $F_{2\alpha}$ metabolite in phase 1 were calculated, they were found to be different in each animal without a recognizable pattern in each of the groups.

The slope of decrease of progesterone during phase 2 was calculated by a linear regression. Animals in each group had similar slopes while the means of slopes for each group were different ($p < 0.01$ by one-way analysis of variance). The control group had the steepest slope in progesterone decrease, followed by the late medroxyprogesterone acetate group, while the early medroxyprogesterone acetate group had the flattest rate of decrease.

When the progesterone slopes for each group of animals were related to the length of dexamethasone infusion a negative correlation was found (Fig. 4), indicating that animals with a rapid drop in progesterone tended to deliver earlier.

Comment

As previously demonstrated, the administration of dexamethasone to the fetus will produce premature labor. The lambs in this study delivered in 45 hours

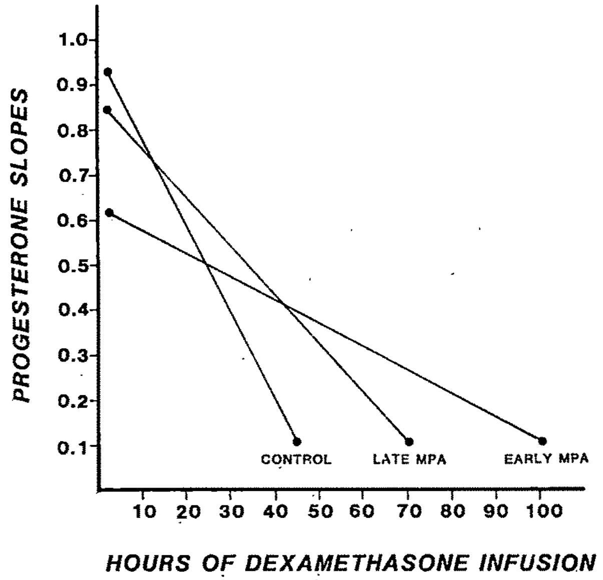

Fig. 4. Relationship between progesterone slopes and hours of dexamethasone infusion until delivery. In each group the decreasing rates of progesterone were 0.936 ± 0.026 for control, 0.805 ± 0.018 for late medroxyprogesterone acetate, and 0.613 ± 0.052 for early medroxyprogesterone acetate (means ± SE in milligrams per milliliter per hour; $p = 0.01$).

rather than 52 hours as in other studies; this could be due to the fact that these sheep were at a more advanced gestational age than in previous studies. We confirmed that endocrinologic events that occur when labor is induced prematurely by dexamethasone infusion are similar to those that are seen when labor occurs spontaneously; however, the events are accelerated.

When medroxyprogesterone acetate was administered to the ewe prior to the start of dexamethasone (early medroxyprogesterone acetate), the onset of labor was delayed and the length of labor was prolonged. The same effect was observed in the group where medroxyprogesterone acetate was administered after the start of dexamethasone infusion (late medroxyprogesterone acetate) but the delay was not as great.

Both treated groups showed a decreased rate of progesterone withdrawal when compared to the control group, the early medroxyprogesterone acetate groups having an even slower rate than the late medroxyprogesterone acetate animals.

When premature parturition is induced by dexamethasone, both estradiol and prostaglandin $F_{2\alpha}$ metabolite increase. It has been postulated that a prostaglandin increase is causally related to the estradiol rise. Thus the estradiol rise should precede the prostaglandin increase. In our experiment this event occurred in two of nine animals. In another six animals the prostaglandin $F_{2\alpha}$ metabolite rise preceded the estradiol rise while in one ewe the rise of the two substances occurred simultaneously.

In sheep, progesterone in large doses has been shown to depress myometrial sensitivity to oxytocin and inhibit labor, whether spontaneous or prematurely induced by fetal administration of dexamethasone or adrenocorticotropic hormone.[14] Progesterone withdrawal will produce significant structural changes in the myometrial gap junction; Garfield et al.[15] demonstrated that progesterone administration can reverse this change.

During parturition, progesterone is metabolized to less active products by the action of 17α-hydroxylase. Barbieri and Ryan's[16] study on rat testicular steroidogenesis demonstrated that medroxyprogesterone acetate inhibits 17α-hydroxylase. A similar mechanism may be operative in the placenta so that administration of medroxyprogesterone acetate may maintain elevated progesterone concentrations for a prolonged time, thus continuing the progesterone block. Another mechanism by virtue of which administration of medroxyprogesterone acetate may delay parturition is by a direct effect of this steroid on the myometrium, that is, medroxyprogesterone acetate may substitute for progesterone in maintaining the progesterone block. This last mechanism does not appear to be operative in our experiment since uterine contractility was related more to the removal of the progesterone block and less to the medroxyprogesterone acetate concentration.

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CORRESPONDENCE

Management of placenta previa

To the Editors:

D'Angelo and Irwin in their paper on conservative management of placenta previa (AM J OBSTET GYNECOL 1984;149:320) have documented that prolonged hospitalization not only benefits the mother, but it also provides the obstetrician with increased peace of mind and, with increased cost-effectiveness, makes good financial sense. One factor that needs to be considered is the false positive rate of placenta previas as determined by a "double set-up." One of the discussants, Dr. Dermot MacDonald, eluded to the fact that in his institution the digital examination has been abandoned in view of the accuracy of sonography. If all patients with suspected placenta previa were hospitalized, the false positive rate would greatly increase the cost of hospitalization. Some state that ultrasonography must be repeated at least 1 week prior to intended delivery in order to delineate and confirm placental implantation. Furthermore, Dr. MacDonald states that in his group of patients that were hospitalized and managed conservatively with placenta previa, seven, or 4%, were delivered vaginally. However, a neonatal mortality of 40% was present in those that were delivered vaginally. Although sonography has reached a great degree of sophistication, we would still support the view that a carefully planned double set-up prior to scheduled delivery is essential. False positive and negative results are frequently seen. Ultrasonography may miss a placenta previa, or a placenta previa may be documented when there is none.

It is our practice to inhibit uterine contractions in cases of placenta previa because of the belief that uterine contractions may precipitate bleeding. This was also found to be true in the majority of cases in the series of D'Angelo and Irwin. Use of tocolysis may add considerable time before an episode of hemorrhage.

A recent report by Sampson et al.[1] detailed the beneficial effects of terbutaline in cases with placenta previa. Although highly desirable, a controlled study of tocolysis and placenta previa as suggested by Dr. Rosen is virtually impossible to accomplish in our current medicolegally oriented society.

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Reply to Semchyshyn and Chervenak

To the Editors:

We agree in principle to the concepts alluded to by Semchyshyn and Chervenak. We would like to take this opportunity to expand on the concept of false positive ultrasonographic diagnosis of placenta previa, however. We have observed several such cases in our institution. Indeed a case did occur during the study period of our report. This index case reflects our experience with this phenomenon. The patient was admitted at 32 to 33 weeks' gestation with a clinical presentation and ultrasonographic findings consistent with marginal placenta previa. Conservative management as described in our report resulted in attainment of 36 weeks of completed obstetric gestation and a mature amniocentesis. Double set-up examination allowed for induction of labor and vaginal delivery of a mature surviving neonate. Gross examination revealed an area of semilunar infarction at the leading border of the placenta. Similar experiences have led us to believe that these cases do not represent true false positive ultrasonographic diagnoses. These patients are at risk for severe maternal hemorrhage necessitating preterm delivery and/or premature labor. We therefore feel cost/benefit criteria are indeed met.

An effort must be maintained to avoid overtreatment of true false positive ultrasonographic diagnoses of placenta previa in a hope to maintain cost-effectiveness of therapy. When ultrasonographic findings and clinical presentation are consistent with placenta previa, we delay continuous hospitalization until a minimum gestational age cut-off is reached. We have used a minimum gestational age for total placenta previa (placentas seen crossing from one edge of the internal os to the other) of 28 weeks' gestation and for marginal placenta previa (edge of placenta at edge of internal os) of 32 weeks' gestation. We have found that these minimum gestational age cut-offs eliminate false positive diagnoses due to further placental migration. Delaying continuous hospitalization until these minimum gestational age criteria are met has resulted in no excess neonatal morbidity while allowing for cost-effectiveness.

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The abdominal pelvic pain syndrome: fact or fiction

To the Editors:

We read with great interest the article on the abdominal pelvic pain syndrome by D. G. Slocumb (AM J OBSTET GYNECOL 1984;149:536) because chronic pelvic pain indeed may be a frustrating condition for both patient and doctor. Great was our elation at reading the introductory statement that at last a neurological and treatable condition had been identified that could account for this enigmatic pain syndrome. There is no doubt in our mind that the results of treatment are true, but we are not convinced therefore that the author has provided the proof either of the neurological nature of this syndrome or the specificity of the therapy. In what percentage of women without chronic pelvic pain can similar trigger points be found? In other words, how specific are these points for patients with pelvic pain? As far as we understand, the treatment was perfectly amenable to a double-blind, placebo-controlled trial (injection of saline or bupivacaine). In a double-blind comparison of mepivacaine injection versus saline injection for myofascial pain, F. A. Frost and co-workers[1] showed that the latter was slightly superior in giving pain relief. The referral to a special clinic where scrupulous attention is being given to the pain problem may be associated with a strong placebo effect, and the needling of trigger points alone without injection of anesthetics can relieve pain.[2] It is not clear from the text which and how many patients received additional medical therapy, varying from antiprostaglandins to antianxiety medications, and how these medications could have affected the results of treatment. Neither is it clear whether all these patients have been subjected to a uniform protocol of gynecologic and other examinations prior to this study.

We agree with Dr. Slocumb that examination for trigger points may be a useful diagnostic and therapeutic addendum in dealing with unexplained pain but before concluding that the pain is unexplained, all pelvic structures should have been investigated by appropriate measures, including laparoscopy. Pain arising from or coexistent with ovarian cysts, endometriosis, or fibroids may be relieved by blocking the trigger points, but this fact does not obviate the need for a more appropriate treatment. Pain relief without an appropriate diagnosis could be life-threatening in the event of an ectopic pregnancy, pelvic inflammatory disease, or pelvic vein thrombosis. Moreover, evidence is lacking in the text to justify the degradation of the universal joint syndrome to a chimera.[3] Pending more convincing data, we cannot take part in the overenthusiasm of Dr. Slocumb and we are afraid that, one day, the abdominal pelvic pain syndrome might join the other figments of our frustration.

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Reply to Dhont et al.

To the Editors:

I want to thank the authors of this letter for pointing out concerns about observations and statements made in my article on chronic pelvic pain. Indeed the concepts and experiences expressed in that article deviate considerably from established therapeutic protocols and theory. For instance, to say that a majority of prolonged abdominal pelvic pain originates from focal tender points in the abdominal wall is most remarkable. How can abdominal wall fat be a cause/source of such severe, diffuse, and enigmatic pain? Although not a common concept in assessment of abdominal pelvic pain, there are numerous references in the literature attesting to consistency in findings viewed from a wide range of specialties.[1-4]

There can be no doubt that the cause of much of pelvic pain is neurological in nature, particularly when 74% of pain clinic patients have the same clinical findings and 89% respond to local infiltration.[5] Proof of the specificity, however, must include appropriate controls, double-blind comparisons, and long-term follow-up. Trigger point infiltration is not a cure but an observed clinical response to alterations in pain thresholds.

It is important, however, to recognize that many interventions in abdominal pelvic pain can alter pelvic pain for varying durations. In our clinic reassurance, acupuncture, laparoscopy, hypnosis, and biofeedback all have produced temporary relief. More significantly, laparotomy and/or hysterectomy have resulted in 3 to 12 weeks of pain relief whether or not the uterus, tubes, or ovaries have been removed. In 31 of our patients who had hysterectomy for pain elsewhere, the same pain returned. In 17 patients in whom trigger point blocks offered only temporary relief with pain returning with next menses, 5 responded to hysterectomy alone and 12 had return of trigger point pain after hysterectomy with 9 responding to subsequent trigger point therapy.[5] Apparently a variety of techniques of altering central, spinal, and peripheral nociceptive function can result in varying degrees of temporary pain relief.

It should be noted that the references in the letter of Frost et al., although documenting pain relief, were of short-term follow-up and, for the most part, temporary relief. For instance, in Frost et al.'s paper[6] on double-blind saline and mepivacaine injections for myofascial pain, only 16% and 18% respectively were pain free after 4 days. In K. Lewit's study[7] of needle effect of myofascial pain, only 58 of 288 sites (20%) had relief for "several months."

Indeed needling and saline injection can alter trigger point pain thresholds. In our clinic, however, saline control infiltration in six pilot patients was not effective in achieving pain relief beyond 72 hours.[8] What is more important, the average duration of follow-up was 17 months in the pain study, with many patients entering their fourth and fifth year of follow-up as of this writing.

Another observation is that myofascial trigger points have different characteristics than do abdominal wall points and are more likely to be primary pain experiences, whereas abdominal wall trigger points often follow pelvic or abdominal trauma.

The authors question the effects of supportive therapy in the overall outcome, a valid observation which is being assessed at this time. Needless to say, 28% of the patients were treated in a non–pain-clinic setting (emergency room, in hospital, general clinic), 14% received narcotics, and 6% received antidepressants for a short term (1 month) after initiation of therapy. A majority (68%) received no supportive medications, many having been withdrawn from previous medication protocols.

The value of trigger point pain diagnosis and therapy in avoiding unnecessary therapy is considerable. At no time has it obscured pain originating from a pathologic process, e.g., appendicitis, ectopic pregnancy, infarcting fibroid. It has been consistent that when all pain can be blocked for a duration in excess of the action of the local anesthetic, the coexistence of benign pelvic processes such as ovarian cysts, fibroids, adhesions, and treated endometriosis are incidental. Evaluation and treatment of their processes should have independent indications if the pain is responsive to trigger point infiltration. Invariably the same pain will exist after surgical therapy, requiring neurological consideration. Obviously, pain unresponsive to infiltration of trigger points needs further assessment.

In conclusion, I would consider the skepticism expressed by the authors of the letter only after they have approached diagnoses and therapy of pelvic pain as outlined.[5, 8] So crucial is the technique of examination, that the concept cannot be appreciated without appropriate attention. Major efforts are being directed to the double-blind protocols and psychological and placebo factors at this time.

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Abdominovaginal delivery in case of impacted head in cesarean section operation

To the Editors:

The paper by Landesman and Graber (Abdominovaginal delivery: Modification of the cesarean section operation to facilitate delivery of the impacted head. AM J OBSTET GYNECOL 1984;148:707) describes a technique that has been used in our hospital for several years when difficulties arise due to an impacted head during a cesarean section. Recently, this technique has been further improved by a bimanual delivery procedure.[1] It was introduced in a particular case in which intravaginal pressure exerted by the assistant proved to be ineffective. Because of the increase in the deflexion of the head, it was not pushed out of the pelvis but forced against the symphysis. This could be avoided by the bimanual technique, i.e., the surgeon himself delivers the head with one hand in the uterus to avoid further deflexion and the other hand in the vagina to press the head up. Since then, bimanual delivery of the head has proved to be an effective method in many other cases. Thus the surgeon can judge the cephalopelvic relationship better, he has an opportunity to correct an adverse attitude, and no time is lost waiting for assistance. However, as in the Marshall-Marchetti procedure, after vaginal introduction of the hand, the sterile drapes have to be renewed.

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REFERENCE

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Self-examination of the cervix in premature labor prevention

To the Editors:

In pregnant women with a history of previous premature birth, a program was begun in which the women palpated their own cervices. Beginning at 14 weeks of gestation, the subjects were instructed to sit at the edge of a seat after voiding, and to palpate their cervix at least twice a day, much as when inserting a diaphragm. Eight pregnant women, having a total of 11 previous deliveries prior to the 34th week, were successfully enrolled in the program. Ten others declined for various reasons. In addition, five women with a cervical cerclage also participated in the program. After 3 years of experience, a number of observations are possible: (1)

Although pregnant women apparently have no hesitation about coitus during pregnancy, many are reluctant to palpate their cervix. (2) Women who continued to work outside the home during the third trimester reported that their cervix was often difficult to palpate upon arising in the morning as it was generally high and posterior but was easier to palpate at the end of the day as it was more anterior, lower, and softer. (3) Every subject came in on at least two occasions, falsely reporting that her cervix had begun to dilate, and all seemed somewhat apprehensive about their ability to determine cervical dilatation. (4) In all subjects without cerclage, their cervix had dilated at least to 1.5 cm by the 32nd week of pregnancy. (5) One subject with a cerclage and three others correctly reported to the emergency room after having noticed that their cervix had begun to silently dilate prior to the 35th week of pregnancy. (6) Four of five subjects accurately reported their cervical dilatation when in labor at term.

Frequent cervical examination is part of the program of most protocols for the prevention of premature birth.[1] Our experience suggests self-examination may increase patient anxiety, but it also appears to be relatively accurate in early detection of the pregnant women with silent dilatation of her cervix. In addition, it would appear that the normal multigravid woman in the third trimester who is working outside the home experiences a descent and at least partial dilatation and effacement of her cervix during the day, which apparently regresses during the night.

Self-examination of the cervix may be beneficial to detect early cervical dilatation in the more tranquil pregnant women. Such a technique avoids the frequent clinic visits required by other programs designed to prevent premature birth.

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REFERENCE

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Use of Hickman catheter during pregnancy

To the Editors:

We read with interest the case report, "Hickman Catheter Use in a Pregnant Patient Requiring Therapeutic Heparin Anticoagulation," which appeared recently in the JOURNAL (Nelson DM, Stempel LE, Fabri PJ, Talbert M. AM J OBSTET GYNECOL 1984;149:461).

The authors reported use of a Hickman catheter for the administration of prophylactic heparin to a patient with a Starr-Edwards mitral valve, from 20 to 36 weeks of gestation. The case was thought to be the first reported in which such a catheter was used during pregnancy.

We are aware of several other cases. Cox et al.[1] and Martin[2] have previously reported the use of Hickman catheters for the administration of parenteral alimentation solutions in malnourished patients during pregnancy without catheter-related complications. At the Stanford Medical Center, in 1981, a patient with acute leukemia during pregnancy underwent Hickman catheter placement, for administration of antineoplastic chemotherapy, antimicrobials, and parenteral alimentation solutions.[3] Catheter placement was complicated by the development of a mediastinal hematoma and unilateral phrenic nerve paralysis.

Recently, we were consulted with regard to a patient with a St. Jude prosthetic valve, who had undergone uncomplicated indwelling (Broviac) catheter placement at 10 weeks for intravenous heparin anticoagulation. She is currently doing well in the early second trimester.

We agree with Nelson et al. that Hickman (or Broviac) catheter placement is occasionally indicated in pregnancy. However, even in expert hands, insertion of central venous catheters has a 5% rate of serious complications, including pneumothorax, hemothorax, and mediastinal hematoma.[4] We are not aware of studies demonstrating that intravenous administration of heparin for prophylactic anticoagulation during pregnancy offers significant advantages over subcutaneous administration, so long as coagulation parameters are carefully monitored. Thus we would generally favor the subcutaneous route if the placement of an indwelling catheter (Hickman or Broviac) would be required to allow intravenous administration.

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Reply to Catanzarite and Argubright

To the Editors:

We appreciate learning of the experience with the use of central venous catheters in obstetric patients described by Drs. Catanzarite and Argubright. In the patient described in our case report, the need for therapeutic anticoagulation was clear but the patient was unwilling to accept the discomforts associated with heparinization by the usual parenteral routes. Placement of the Hickman catheter permitted therapeutic anticoagulation with heparin and was tolerated very well by the patient. As we noted in our paper, we do not recommend the placement of a Hickman catheter for the majority of pregnant patients requiring therapeutic heparinization since most patients can indeed be managed by parenteral heparin administration without requiring the invasive procedure of placement of a central venous catheter.

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Use of ritodrine in uterine inversion

To the Editors:

Drs. Kovacs and DeVore (Kovacs BW, DeVore GR. Management of acute and subacute puerperal uterine inversion with terbutaline sulfate. AM J OBSTET GYNECOL 1984;150:784) presented two cases of uterine inversion in which the bolus injection of 0.25 mg of terbutaline sulfate resulted in uterine relaxation and facilitated replacement. The following supports the use of such agents in cases of uterine inversion.

A 21-year-old woman, gravida 3, para 0, abortions 2, underwent spontaneous delivery of a term infant. Following delivery of the placenta, a complete uterine inversion was noted. The uterus promptly contracted and could not be replaced manually. As terbutaline was not available, the patient received a bolus injection of ritodrine, 0.150 mg intravenously. Within 15 seconds the pulse rose from 108 to 158 bpm. Initial uterine relaxation was noted at 30 seconds, with maximal relaxation at 90 seconds. The uterus was easily replaced and oxytocin infusion begun, with prompt uterine contraction. Estimated blood loss was 1100 ml. There was no further significant uterine bleeding.

This case illustrates two essential points. First, since terbutaline is rarely available on a labor and delivery unit, the clinician should realize that the more widely available ritodrine hydrochloride may be used safely and with similar effect. Second, in the above case, as well as cases of intrapartum hypertonus, I have found a much smaller dose of sympathomimetic agent extremely effective in inducing uterine relaxation. An amount of 0.150 mg of either agent is effective and will have fewer maternal side effects than a 0.25 mg dose.

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Systemic absorption of metronidazole

To the Editors:

Dr. Richard L. Sweet, in a letter to the Editors (Sweet RL. Reply to Rivera y Almodovar AM J OBSTET GYNECOL 1984;149:807) stated: "Vaginal metronidazole is not absorbed and has only a topical effect against Trichomonas."

Systemic absorption of metronidazole after vaginal administration has been quantified. Opperman et al.[1] reported that a peak plasma concentration of 2.4 μg/ml was reached at 24 hours after vaginal administration of 500 mg of metronidazole. Ralph[2] stated that systemic absorption from the vagina is slower than from other routes with 1.63 μg/ml being attained 8 to 24 hours after a 500 mg dose.

Although these levels are lower than those resulting from oral or rectal administration,[1-3] they are still substantial.

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ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Specialty Review in Neonatology/Perinatology, August 26-30, 1985, Chicago, Illinois. For further information contact: The Cook County Graduate School of Medicine. 707 South Wood St., Chicago, IL 60612, Attn: Dick Nelson. Tel.: (312) 633-2600.

Adolescent pregnancy: Problems & Solutions, May 17, 1985, The Palm Beach Airport Hilton International Ballroom, 150 Australian Ave., West Palm Beach, FL 33402. For more information contact: Becky Myers, Planned Parenthood, 5312 Broadway, West Palm Beach, FL 33407. Tel.: (305) 848-6300.

Practical Management of Infertility, March 29-30, 1985, Ramada Hotel Ballroom, Tyler, Texas. For additional information call: Mr. Howard Kaplan, Serono Symposia, U.S.A. Tel.: (800) 225-5185.

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CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. Risk of Dose: In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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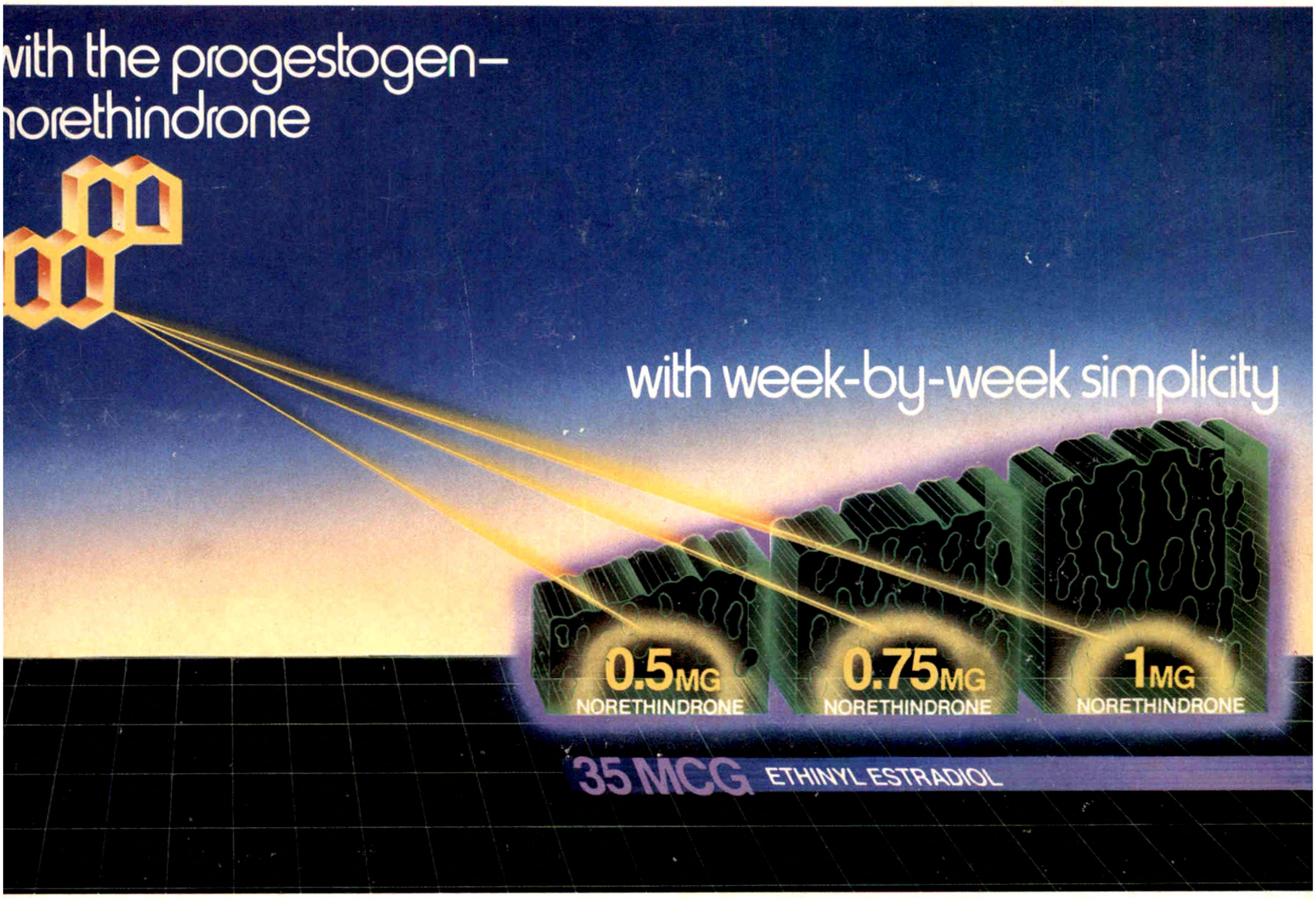

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Reference: 1. Robertson WH: Vulvovaginal candidiasis treated with clotrimazole cream in seven days compared with fourteen-day treatment with miconazole cream. *Am J Obstet Gynecol* 132:321-323, 1978.

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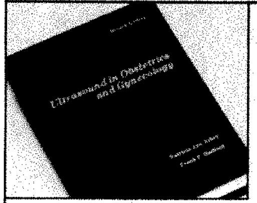

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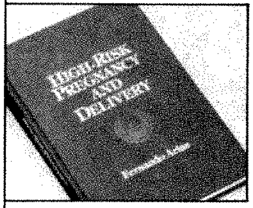

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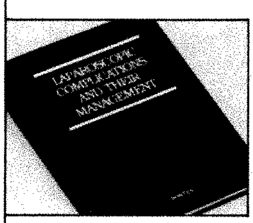

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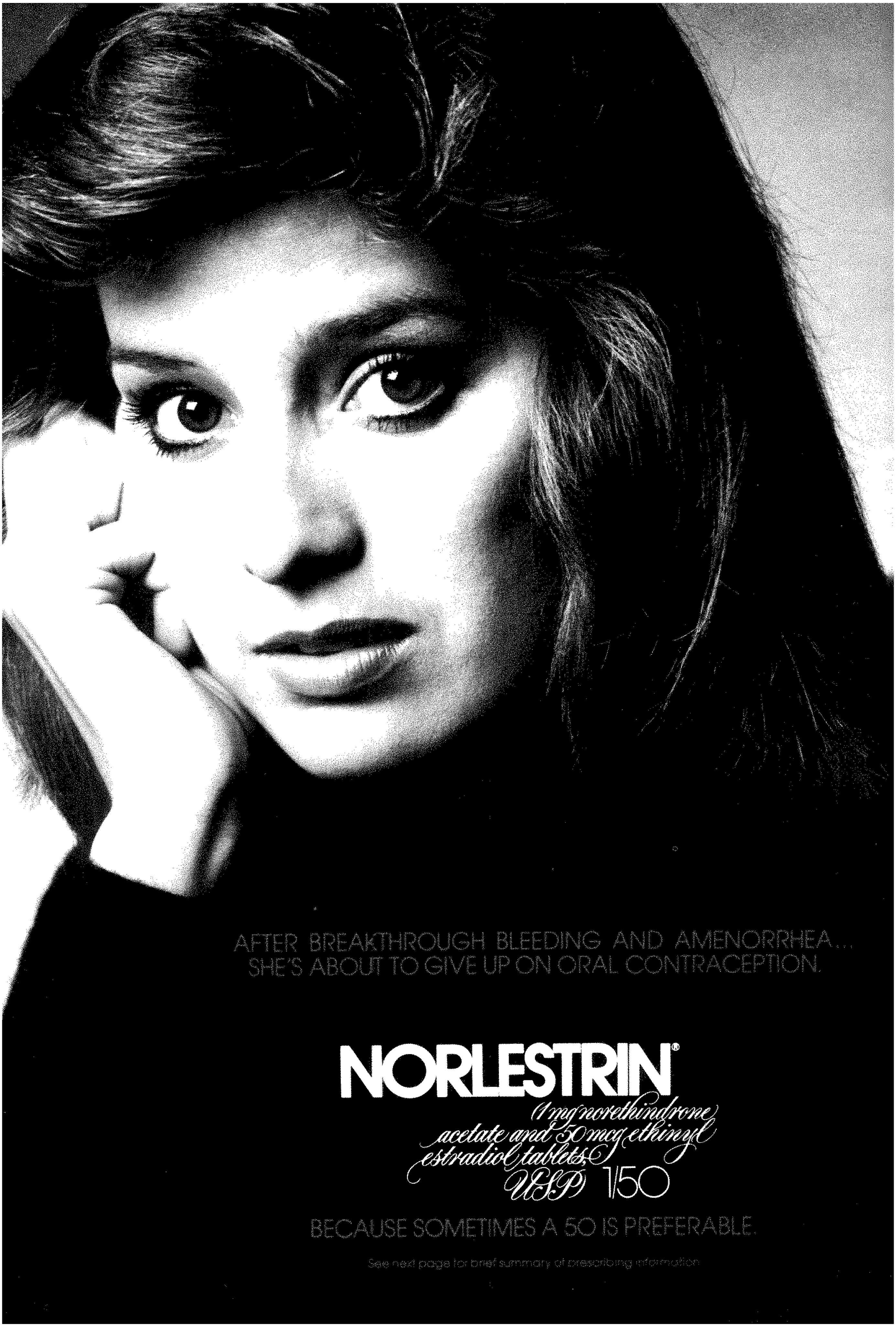
AFTER BREAKTHROUGH BLEEDING AND AMENORRHEA...
SHE'S ABOUT TO GIVE UP ON ORAL CONTRACEPTION.
NORLESTRIN®
(1 mg norethindrone acetate and 50 mcg ethinyl estradiol tablets, USP) 1/50
BECAUSE SOMETIMES A 50 IS PREFERABLE.
See next page for brief summary of prescribing information.

Brief Summary of Prescribing Information

NORLESTRIN® (norethindrone acetate and ethinyl estradiol tablets, USP)

See section under **Special Notes on Administration and HOW SUPPLIED.**
Before prescribing, please see full prescribing information. A Brief Summary follows.

DESCRIPTION

Norlestrin Products are progestogen-estrogen combinations.

INDICATIONS AND USAGE

Norlestrin Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives as a method of contraception.

In clinical trials with Norlestrin 1/50 involving 25,983 therapy cycles, there was a pregnancy rate of 0 05 per 100 woman-years; in clinical trials with Norlestrin 2 5/50 involving 96,388 cycles, there was a pregnancy rate of 0.22 per 100 woman-years.

Dose-Related Risk of Thromboembolism from Oral Contraceptives: Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance

CONTRAINDICATIONS

1 Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5 Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See WARNING No. 5)
8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. The risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks

1. *Thromboembolic Disorders and Other Vascular Problems*. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

Cerebrovascular Disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4 0 to 9.5 times greater

Myocardial Infarction: An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor

Risk of Dose: In an analysis of data, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved

Persistence of Risk: Two studies have suggested that an increased risk may persist for as long as 6 years after discontinuation of oral contraceptive use for cerebrovascular disease and 9 years for myocardial infarction. In addition, a prospective study suggested the persistence of risk for subarachnoid hemorrhage

Estimate of Excess Mortality from Circulatory Diseases: The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. *Ocular Lesions*. Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision, onset of proptosis or diplopia; papilledema; or retinal vascular lesions

3 *Carcinoma*. Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care

4. *Hepatic Tumors*. Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time

5. *Usage in or Immediately Preceding Pregnancy; Birth Defects in Offspring, and Malignancy in Female Offspring*. During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy

There is some evidence that triploidy and possible other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy

6 *Gallbladder Disease*. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. *Carbohydrate and Lipid Metabolic Effects*. Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives

An increase in triglycerides and total phospholipids has been observed.

8 *Elevated Blood Pressure*. An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure

9 *Headache*. Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10 *Bleeding Irregularities*. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11 *Ectopic Pregnancy*. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12 *Breast-Feeding*. Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

PRECAUTIONS

1 A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.

2 Preexisting uterine leiomyomata may increase in size

3 Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

4 Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated

5 Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued

6 Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function

7 Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency

8 Serum folate levels may be depressed.

9 The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted

10 Certain endocrine and liver function tests and blood components may be affected

(a) Increased sulfobromophthalein retention (b) Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin 3; increased norepinephrine-induced platelet aggregability (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone (d) Decreased pregnanediol excretion (e) Reduced response to metyrapone test

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, tetracycline, and ampicillin

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives: thrombophlebitis; pulmonary embolism, coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, eg, retinal thrombosis and optic neuritis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms; breakthrough bleeding; spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes, change in weight; change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine; increase in size of uterine leiomyomata; rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis; change in corneal curvature, intolerance to contact lenses

The following adverse reactions have been reported and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido, chorea; changes in appetite, cystitis-like syndrome, headache; nervousness; dizziness; hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum; hemorrhagic eruption, vaginitis, porphyria

Special Notes on Administration

Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

After several months on treatment, bleeding may be reduced to a point of virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

HOW SUPPLIED

Norlestrin $\boxed{21}$ 1/50 is available in compacts each containing 21 tablets. Each tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills

Norlestrin $\boxed{21}$ 2 5/50 is available in compacts each containing 21 tablets. Each tablet contains 2 5 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills

Norlestrin $\boxed{Fe}$ 1/50 is available in compacts each containing 21 yellow tablets and 7 brown tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate, USP. Available in packages of five compacts and packages of five refills

Norlestrin $\boxed{FE}$ 2.5/50 is available in compacts each containing 21 pink tablets and 7 brown tablets. Each pink tablet contains 2.5 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate, USP. Available in packages of five compacts and packages of five refills.

Norlestrin $\boxed{28}$ 1/50 is available in compacts each containing 21 yellow tablets and 7 white inert tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills.

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American Journal of Obstetrics and Gynecology

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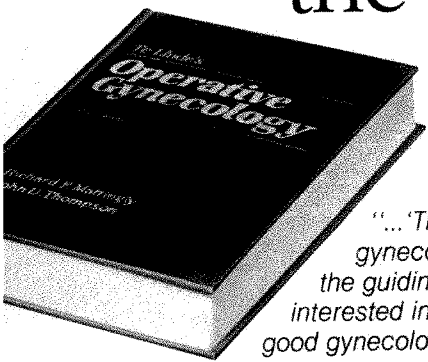

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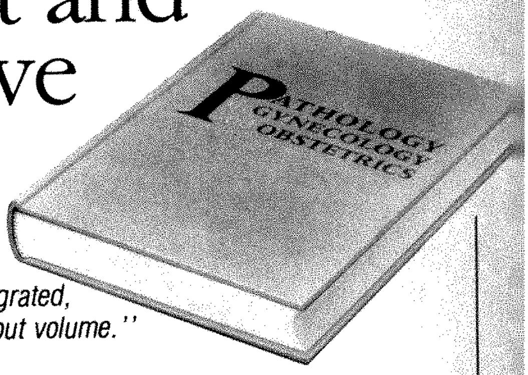

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Precautions: Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Interference with Laboratory Tests—High concentrations (>100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST[‖] reagent tablets.

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Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

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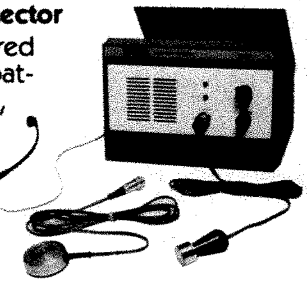

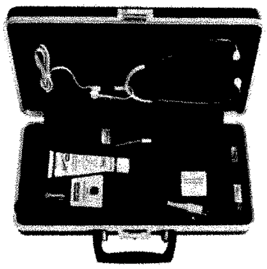

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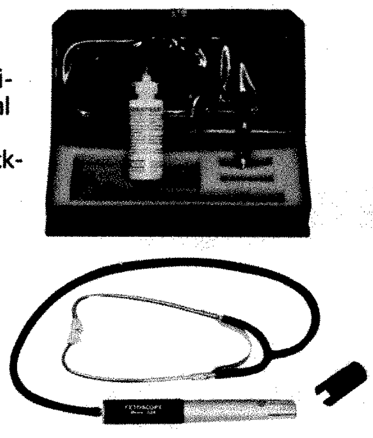

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CLINICAL SECTION

Clinical Opinion

Six myths about controlled trials in perinatal medicine

Robert L. Bryce, M.B., B.S., and Murray W. Enkin, M.D.
Hamilton, Ontario, Canada

Despite a historical commitment to evaluation of the results of practice, obstetrics has missed many opportunities to assess new therapies adequately prior to their widespread use. Although randomized controlled trials are generally recognized as the best method of evaluating therapy, a number of myths about these trials have impeded both their performance and the implementation of their results. The unscientific nature of these myths is discussed, and examples from the perinatal literature are presented. (AM J OBSTET GYNECOL 1985;151:707-10.)

Key words: Controlled trials, perinatal medicine

Obstetrics, among all the clinical specialties, has been foremost in its willingness to examine the results of its clinical activities. Confidential enquiries into maternal deaths and the meticulous recording of perinatal mortality and its correlates have long been a conspicuous feature of our heritage, a history of which we can be justifiably proud.

It was a shock, then, to find that Cochrane,[1] Director of the British Medical Research Council Epidemiology Unit, in assessing the scientific approach of the various clinical specialties, awarded obstetrics and gynecology the "wooden spoon" for the opportunities we had missed by introducing procedures such as Papanicolaou smears and electronic fetal monitoring into widespread practice without prior evaluation by means of properly conducted clinical trials.

Although other designs are frequently used, the randomized controlled clinical trial is recognized as the best way to determine the efficacy and effectiveness of a new treatment or to reexamine the usefulness of current therapy. How can it be that we missed the opportunities in the past to use the information available from controlled clinical trials to aid us in our practice, despite our obvious sincere interest as obstetricians in improving the results of our care?

Many reasons for this have been given, some of them undeservedly unflattering. A principal reason may be that as practical clinicians we tend to rely on our own experience and that of our mentors. We feel uneasy about the results of clinical research that sometimes appear to contradict our own personal observations. That unease quite understandably may result in attempts to discredit the value of the randomized controlled clinical trial. Chalmers[2] has previously addressed some of the strategies used to discredit the prospective experiment. The antipathy toward the concept of these trials has been bolstered by a number of widely held myths. In this paper we will discuss and attempt to dispel six of these myths (Table I).

Myth 1: There is nothing special about a controlled clinical trial

Clinical decision-making is usually based on evidence, but the quality of that evidence is not always as strong as we would like it to be. An understanding of underlying mechanisms and personal experience are useful guides to therapy but are not enough. Treatments based on a plausible scientific basis alone have too often been proved useless or harmful when subjected to empirical test. Even the most noted experts in a field can be misled by biases of which they are unaware.

In observational studies, results for a group of pa-

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Table I. Six myths about controlled trials in perinatal medicine

1. There is nothing special about a controlled clinical trial
2. Randomization is unethical
3. You can prove anything with statistics
4. Trials damage our doctor-patient relationship
5. No one pays any attention to the results of clinical trials
6. Someone else will do the trials

tients treated in a certain manner are reviewed. Although no concurrent group of similar patients who were treated differently is identified, the treated group is (often only implicitly) compared either to previous experience or to patients who did not receive the therapy under study. Unfortunately, neither of these comparisons is satisfactory. We can never be sure that any differences observed were not due to extraneous factors. Patients change, as do other aspects of their care.

The absence of a concurrent, comparable control group leads, in general, to an overly optimistic assessment of benefit. In a review of the literature on treatments for respiratory distress syndrome in preterm infants, Sinclair[3] found that although 89% of uncontrolled studies claimed a benefit from the proposed therapies, only 50% of the controlled studies appeared to show a benefit. These findings are not surprising given the potential for bias inherent in uncontrolled studies.

The solution to this problem is to provide a control group of patients who are, to the maximum extent possible, identical to those who are going to receive the experimental therapy but who receive either a placebo or standard therapy in its stead. At the end of the study, one then compares clinically relevant outcomes for those who received the experimental therapy with outcomes for those who did not. The special feature of the controlled clinical trial, unlike uncontrolled observations, however extensive, is that it allows considerably more confidence that the differences observed were actually due to the intervention rather than to coincidental, or associated, factors.

Myth 2: Randomization is unethical

Some doctors feel uncomfortable about randomization because patients allocated to the control group are being denied the potential benefits of the newest, and presumed best, therapy. This argument has recently been leveled in Britain against a large randomized controlled trial of periconceptional vitamins administered to mothers who had previously given birth to an infant with a neural tube defect, to consider its effectiveness in the prevention of recurrence. A nonrandomized trial by Smithells et al.[4] had compared the incidence of recurrent neural tube defect in the offspring of mothers who were receiving a multivitamin preparation at the time of conception with that in offspring of mothers in a control group who were not receiving such vitamins. There was a much higher incidence of recurrent neural tube defect in the control group. Many believed that the value of periconceptional vitamin administration was established by this trial, and it was now time for implementation of vitamin therapy rather than for further investigation.

Closer examination of the composition of the two groups, however, reveals important differences between them. Fifty percent of the control mothers were Irish, as opposed to 20% of the treatment group. This is relevant, as the incidence of neural tube defect is much higher in Ireland than in other countries. Additional recognized or unrecognized differences between those women who chose to use the vitamins and those who did not might also have influenced the resultant incidence of recurrent neural tube defect. It could well be that the differences in outcome attributed to the vitamins could in fact be due to inherent differences between the two groups rather than an effect of the therapy. Randomization would have helped those differences to be more evenly distributed between the groups, thus allowing the treatment effect (if any) to become evident.

In addition, therapy instituted with the best of intentions may have unsuspected adverse effects. The risks of periconceptional vitamins to the developing fetus are unclear, as was the risk of diethylstilbestrol when controlled clinical trials of this drug in pregnancy were carried out.[5] At the time of the diethylstilbestrol trials, women may have feared that to be in the control group would have deprived them of the presumed benefits of the medication. There can be little doubt that those who were in the control group were thankful that they did not receive the active medication when the harmful effects of diethylstilbestrol became known.[6] Although randomization may deny potentially beneficial therapy to control patients, it also protects them from potentially harmful therapy.

The first ethical imperative of any experiment is that it has the potential to answer the question it sets out to answer. If it does not have this potential, then any hazard, discomfort, or expense suffered by the subjects or by society as a whole, however slight, is unjustified. A trial in which every effort is not made to ensure that the control group is comparable to the group receiving the therapy under investigation does not have this potential. Failure to randomize when possible is inherently unethical.

Myth 3: You can prove anything with statistics

It has been said that there are "lies, damn lies, and statistics" and that the reason for statistics is that "lies and damned lies are not good enough." Clinicians, with conventional medical backgrounds, have an understandable fear of numbers. We are inherently suspicious of the manipulation of figures and often feel that statistics are really a form of deception, a way of bewildering us with complex mathematics and obscure jargon.

It is good that we regard published statistics with caution. Nevertheless, the ability to quantitate effects is essential to the evaluation of any form of therapy. A basic understanding of statistical principles is sufficient to overcome our fears and allow us to read and critically evaluate the data in published reports. There are three simple questions we should ask in the statistical evaluation of a study of any therapy.

1. If a difference in outcome between the treatment and control group was observed, is it likely that the difference was due to the treatment, or could it have arisen by chance alone?

The likelihood that the difference observed was due to chance is usually expressed as a probability or "p value." The levels of significance of these probabilities are conventionally set as greater or less than 0.05 or 0.01. These levels are guides to interpretation but are not strict rules.[7] We should be suspicious of even large observed differences if the probability that the difference may have been due to chance was not calculated and reported.

2. If no difference is found between the treatment and control groups, was the sample size large enough to have detected a difference if one was really present?

In a review of 71 "negative" randomized controlled trials published in major medical journals, Freiman et al.[8] demonstrated that because of inadequate sample sizes 67 of these trials had a >10% risk of missing a true 25% therapeutic improvement. With the same risk, 50 of these trials could have missed a 50% improvement. For example, more than 100 observational studies of electronic fetal heart rate monitoring failed to establish the benefits of this modality.[9] Five small randomized trials, totaling 2922 patients in all, individually failed to show any differences in mortality or morbidity between infants electronically monitored and those monitored by intermittent auscultation although statistically combining them showed a small decrease in neonatal convulsions.[10] It required a trial of more than 13,000 patients* to demonstrate a significant difference in morbidity between the two groups (more neonatal convulsions in the intermittent auscultation group). The enormous cost of the other studies could have been saved had the appropriate trial been done originally.

3. Finally, if a statistically significant difference was found, was it large enough to be clinically important? A difference, to be a difference, must make a difference.

With a large sample size, small and meaningless differences between groups can be statistically significant. The National Collaborative Perinatal Project was a large cohort analytic study with follow-up of 54,000 children from birth to 7 years of age. When the data for white male subjects are used as an example,[11] a highly statistically significant difference ($p < 0.001$) in mean Intelligence Quotient at age 7 was found between those children with Apgar scores of 0 to 3 at 1 minute and those with Apgar scores of 8 to 10. However, the difference in Intelligence Quotient between the two groups was only 3.5, which is clinically unrecognizable and of doubtful importance.

Statistics "prove" nothing. They do, however, enable us to estimate the probability that the results observed occurred by chance alone. Our clinical understanding can help us to determine the relevance of these results to our practices and to our patients.

Myth 4: Trials damage our doctor-patient relationship

Patients require confidence in their doctors and respond better to treatment when they do have confidence in their doctors. A belief in the efficacy of what we prescribe is an important component of therapy. In the words of Asher,[12] "If you admit to yourself that the treatment you are giving is frankly inactive, you will inspire little confidence in your patients unless you happen to be a remarkably gifted actor, and the results of your treatment will be negligible. But if you can believe fervently in your treatment, even though controlled tests show that it is quite useless, then your results are much better, your patients are much better, and your income is much better, too."

However, the powerful therapies available today have a great potential for harm as well as good. While a confident, authoritarian approach can be comfortable for both doctors and patients, it may be a dangerous luxury. Few honest physicians can maintain delusions of omnipotence or deny having frequent doubts. If the relationship with our patients precludes doubt, we can never learn whether the treatment we are administering is really the best possible.

If there is no doubt, then a clinical trial is clearly inappropriate. However, if any doubt exists, the questions in our minds can frequently best be answered by a clinical trial. The purpose of a trial is not to prove that a therapy is, or is not, effective but to find out whether it is or is not effective. Admitting our doubts, and using the best possible means to resolve them, may alter the traditional doctor-patient relationship, but it can make that relationship less paternalistic, more honest, and, in the long run, more effective.

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*Macdonald D, Grant A, Pereira M, Boyland P, Chalmers I. The Dublin randomized controlled trial of intrapartum electronic fetal heart rate monitoring. Presented at the twenty-third British Congress of Obstetrics and Gynaecology, London, 1983.

Myth 5: No one pays any attention to the results of clinical trials

Clinical practice is determined by many factors in addition to scientific evidence. Acceptability, feasibility, and conservatism can influence or impede the implementation of the findings of controlled trials. We have often failed to act on the information provided by clinical trials, to the detriment of our patients. A randomized controlled trial as long ago as 1922 failed to demonstrate that perineal shaving prevented infection,[13] yet this practice has continued to the present day in many centers. Diethylstilbestrol, introduced in 1947 without prior clinical trials for the prevention of miscarriage, remained in widespread use until the mid-1960s, despite randomized controlled trials which failed to confirm any of its professed effects.[5] Much later, only after the demonstration of its tragic potential for adenocarcinoma of the vagina[6] and other anomalies in the offspring of women who had used this ineffective drug during pregnancy, was the use of the drug phased out.

However, on the firm basis of clinical trials, some useless or harmful therapies have been eliminated. The use of unlimited oxygen for premature infants was discontinued when a randomized controlled trial demonstrated it to be associated with the development of retrolental fibroplasia.[14] Other therapeutic innovations have been introduced and incorporated into routine management, for example, antenatal anti-D immunization of rhesus-negative mothers[15] to prevent rhesus isoimmunization.

Although their potential effect as guides to rational therapy has not yet been fully realized, controlled trials have already had a profound effect on clinical practice. A register of controlled clinical trials in perinatal medicine is maintained by the National Perinatal Epidemiology Unit in Oxford.

Myth 6: Someone else will do the trials

The prime concern of a clinician is the care of each individual patient. There is little energy, and even less time, left over to address broader research questions. However, unless perinatal research is carried out by those actively involved in perinatal care, the questions most relevant and important will remain unanswered. When practicing clinicians become more actively involved in clinical trials, research will be directed toward the clinically important questions and will be presented in a form directly relevant to clinical decision-making. If we don't do it, who will?

Comment

We have discussed six widely held myths surrounding clinical trials. Each has been used as an emotional argument against the performance of trials or the acceptance of their results. However, if, as clinicians, we become involved in the planning, performance, and interpretation of clinical trials, we can all play our vital role in evaluating the treatments we are now using, as well as new therapies in the future. All that we need to begin is to admit doubt.

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Perinatal complications in group B streptococcal carriers: A longitudinal study of prenatal patients

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Although prenatal group B streptococcal detection and eradication have been proposed to prevent morbidity, the risk of perinatal complications in prenatal carriers of group B streptococci has not been defined. We evaluated 718 prenatal patients with serial cultures to compare morbidity in carriers and noncarriers. Complications occurring more frequently ($p \leq 0.05$) in prenatal carriers were: (1) collective morbidity, (2) low birth weight, and (3) premature rupture of membranes associated with low birth weight. Maternal pelvic infection and neonatal sepsis were increased in colonized women at delivery but not in prenatal carriers. Ninety-two percent of colonized women were not delivered of low birth weight infants. No carriers delivered vaginally or by repeat cesarean section became infected. Neither inoculum size nor chronic carriage was related to morbidity. The predictive value of a positive prenatal culture did not exceed 8% for any of the complications. We concluded that overall morbidity in carriers of group B streptococci is greater than in noncarriers; however, the risk for specific complications is too low to justify routine testing for detection of group B streptococci until prospective study demonstrates the value of such programs. (AM J OBSTET GYNECOL 1985;151:711-7.)

Key words: Infection, group B streptococci, culture, sepsis

Concern for group B streptococcal morbidity began between 1958 and 1968 when reports of serious neonatal infections appeared in the literature and group B streptococcal meningitis was described for the first time.[1] During the 1970s, interest was focused primarily on neonatal sepsis because these infections were severe, were sometimes misdiagnosed as respiratory distress syndrome, and were associated with a mortality rate >50%.[2] Recent data suggest that the spectrum of group B streptococcal perinatal complications extends beyond neonatal infections. Premature rupture of the membranes and preterm delivery have been reported to be more common in women colonized by group B streptococci on admission for delivery.[3] These women may also be at risk for infection. If delivery is by cesarean section, pelvic infection has been found to be more frequent and more severe.[4]

A new perspective is evolving regarding the risk of morbidity in pregnancies associated with group B streptococcal colonization. This raises new questions concerning potential benefits of group B streptococcal antepartum screening cultures. When neonatal sepsis was the only concern, such programs were not indicated because of the poor correlation between maternal group B streptococcal colonization and newborn infection.[5] If group B streptococcal carriers are also at risk for preterm delivery and maternal infection, identifying and treating such women during pregnancy may be indicated. The merit of such programs depends on several factors. One is the extent to which morbidity is increased in prenatal group B streptococcal carriers compared to noncarriers. Another is the predictive value of an abnormal test, that is, the fraction of patients with a positive group B streptococcal prenatal culture who subsequently have a perinatal complication. These issues have not been resolved because previous studies focused only on morbidity associated with group B streptococcal carriage at delivery. Since prenatal group B streptococcal colonization is characterized by a high rate of spontaneous loss and acquisition,[6] conclusions based on recovery of group B strepto-

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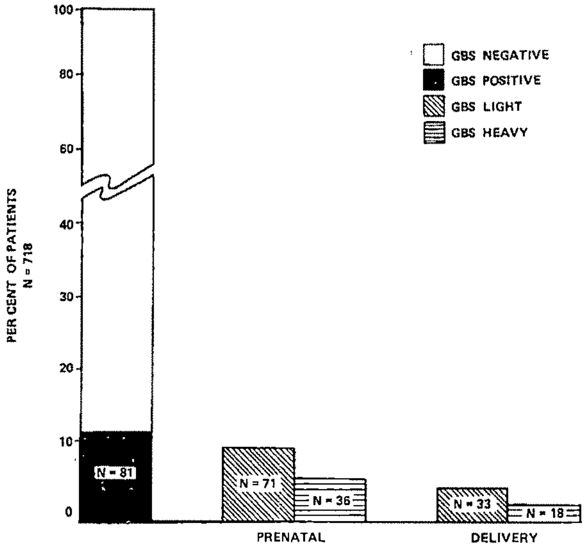

Fig. 1. Frequency of group B streptococcal colonization and inoculum size during pregnancy and at delivery. Mean number of prenatal cultures per patient: negative = 3.9, positive = 4.2. First prenatal culture positive = 6.4% (46 of 718).

cocci at delivery may not apply to prenatal populations.

The purposes of this investigation were to determine, in a longitudinal study, if perinatal complications are more frequent in group B streptococcal carriers (prenatal and/or at delivery) than in noncarriers, to establish the predictive value of positive prenatal group B streptococcal cultures, and to determine if inoculum size and long-term presence of the group B streptococcal carrier state affect maternal and neonatal morbidity. To assure that observations would not be invalidated by clinical intervention, antepartum culture results were not available to physicians. Also the administration of antibiotics was limited to patients with clinical evidence of infection. Antibiotic prophylaxis was not used at cesarean section in this investigation.

Material and methods

The study was done at Fitzsimons Army Medical Center, Aurora, Colorado. The purpose and nature of the study was discussed with prenatal patients and those consenting to participate signed an informed consent. Vaginal cultures were planned for the initial prenatal visit, at 24 weeks, then every 4 weeks, and on admission for labor and delivery.

The culture sites were the lateral and posterior vaginal fornices. Specimens were immediately inoculated to a 5% sheep blood agar plate and *Streptococcus* test broth (Todd-Hewitt broth containing 5% sheep blood, 0.025 µg/ml of neomycin, and 0.0125 µg/ml of nalidixic acid). Prenatal specimens were taken by one of three clinicians and delivered to the laboratory within 2 hours of collection. Specimens collected on admission for labor and delivery were placed in an incubator until the following morning. Plates inoculated in the clinic were streaked in the laboratory whereas specimens taken on admission for labor and delivery were streaked by the admitting physician. On receipt in the laboratory, the *Streptococcus* test broth was immediately subcultured to a blood agar plate and a Columbia neomycin, naladixic acid plate (neomycin, 5.0 µg/ml; naladixic acid, 2.5 µg/ml). The blood agar plate was incubated at 37° C with 5% carbon dioxide and examined at 24 to 48 hours. The Columbia plates were incubated anaerobically and examined after 48 hours' incubation. The *Streptococcus* test broth was incubated for 24 hours, subcultured, and reexamined as described above. Suspected Lancefield group B streptococci isolates were subsequently identified by their characteristic β-hemolysis, yellow pigmentation production on anaerobic Columbia agar,[7] positive sodium hippurite hydrolysis,[8] and Camp test.[9]

The procedure for quantitating cultures was as previously described.[10] In this report, growth of group B streptococci in broth only is termed "light." Growth on the blood agar plate, at the site of initial inoculation or on subsequent streaks, was termed "heavy" colonization. Culture reports were not made available to the attending physicians and were not placed on the patient's chart.

Pelvic infection associated with cesarean section was managed by early recognition and treatment rather than with prophylactic antibiotics. The administration of antibiotics was, therefore, restricted to patients with clinical evidence of infection.

Maternal and newborn records were reviewed after discharge. The diagnosis of pelvic infection was based on a temperature elevation of $\geq$100.4° F during labor and/or post partum, a clinical impression of pelvic infection, and treatment with parenteral antibiotics. The diagnosis of neonatal sepsis was based on the neonatologist's clinical diagnosis, "neonatal sepsis suspected, not proved—treated." None of these newborn infants had positive body fluid cultures. Infants weighing $<$2500 gm were considered low birth weight. The diagnosis of premature rupture of the membranes was made if membranes ruptured prior to the onset of labor. The presence of premature rupture of the membranes was determined only in low–birth weight infants.

Comparison of the significance of the differences in morbidity was evaluated with the use of Fisher's exact test. Reported p values are two-tailed.

Results

Frequency of positive group B streptococcal cultures. A total of 937 pregnant women were enrolled in

Table I. Relationship of inoculum size to perinatal complications in group B streptococcal carriers

	Prenatal carrier					*Carrier at delivery*				
	Light (n = 71)		*Heavy (n = 36)*			*Light (n = 33)*		*Heavy (n = 18)*		
Perinatal complication	*No.*	*%*	*No.*	*%*	*p*	*No.*	*%*	*No.*	*%*	*p*
Pelvic infection	5	7	3	8	NS	4	12	1	6	NS
Pelvic infection per number of primary cesarean sections	5/7	71	2/2	100	NS	4/4	100	1/1	100	NS
Delivery of low–birth weight infant	6	8	1	3	NS	2	6	2	11	NS
Premature rupture of membranes and delivery of low–birth weight infant	4	6	1	3	NS	2	6	2	11	NS
Suspected neonatal sepsis	5	7	2	6	NS	5	15	2	11	NS

the study; 195 dropped out for a variety of reasons (transferred, discharged, aborted, changed their minds regarding participation, etc.), 14 laboratory reports were lost, and 10 hospital charts could not be found for review. The remaining study population totaled 718 pregnant women. The frequency of positive group B streptococcal cultures is shown in Fig. 1; 11.3% of patients (81 of 718) had at least one positive group B streptococcal culture during pregnancy or at delivery. Prenatal group B streptococcal isolation, light growth (71 of 718, 9.9%) or heavy growth (36 of 718, 5%), was twice as frequent as at delivery (33 of 718, 4.6%, and 18 of 718, 2.5%, respectively). These differences were expected because serial cultures were performed in the prenatal group and compared to the single specimen at delivery. According to our bias, a predominance of perinatal complications would occur in patients heavily colonized during pregnancy and at delivery.

Effect of group B streptococcal inoculum size on perinatal complications. To determine if heavy group B streptococcal colonization influences morbidity the frequency of perinatal complications was compared in relation to the inoculum size (Table I). For each of the complications the risk was no greater in heavily than in lightly colonized patients, either prenatally or at delivery. The data, therefore, did not support our preinvestigation bias. Identification of heavy group B streptococcal colonization during pregnancy was not associated with an increased risk of perinatal complications.

Effect of chronic presence of group B streptococcal carriage on perinatal complications. Longitudinal study of group B streptococcal carriage in pregnancy indicates that colonization is often inconstant.[5] A question not previously addressed is the relationship of perinatal complications to the chronic presence of the carrier state. We classified prenatal group B streptococcal carriage as chronic, transient, or intermittent and defined such patients as follows: (1) Chronic carriers were women who had a minimum of three positive prenatal group B streptococcal cultures; (2) transient carriers had three or more prenatal cultures with group B streptococci isolated only once (prior to or at delivery); and (3) intermittent carriers were patients who could not be placed in either of these two groups.

The frequency of complicated pregnancy in the above-classified patients is shown in Fig. 2. Although the risk of morbidity appears to be increased in chronic carriers when compared with transient carriers (26%, six of 23, versus 9%, three of 33, respectively), we believe that impression may be invalid for two reasons. First, our classification may be spurious since 31% (25 of 81) of the preterm group B streptococcal population were intermittent carriers and 43% (seven of 16) of morbid pregnancies were in that group. Second, we could detect no pattern in the perinatal complications seen in chronic carriers. Only two of the six chronic carriers were delivered of low–birth weight infants and only one of those had premature rupture of the membranes. At delivery, three had positive cultures, two negative, and one did not have a culture taken.

Interestingly, 13.3% (10 of 75) of the group B streptococcal carriers who had at least two antepartum cultures taken had positive cultures only at delivery. Although 19% (three of 16) of the group B streptococci–related complications were in this group, serial prenatal screening did not identify patients at risk.

Frequency of complicated pregnancies. When perinatal complications were considered collectively, morbidity was more frequent in group B streptococcal carriers than in noncarriers (Fig. 3). This was true whether the group considered was (1) women at 28 weeks' gestation (29%), (2) prenatal patients with one or more positive cultures (18%), (3) women colonized only at delivery (24%), or (4) all women who ever had positive cultures (21%).

Pelvic infection and primary cesarean section. The frequency of pelvic infection was greater ($p < 0.05$) in

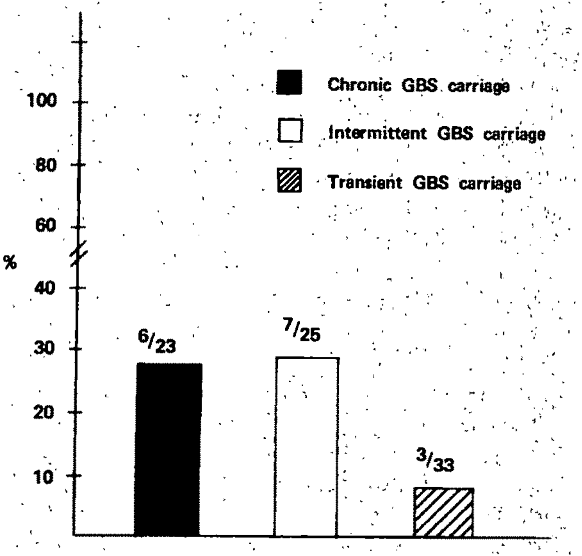

Fig 2. Frequency of complicated pregnancy in chronic, intermittent, and transient group B streptococcal carriers.

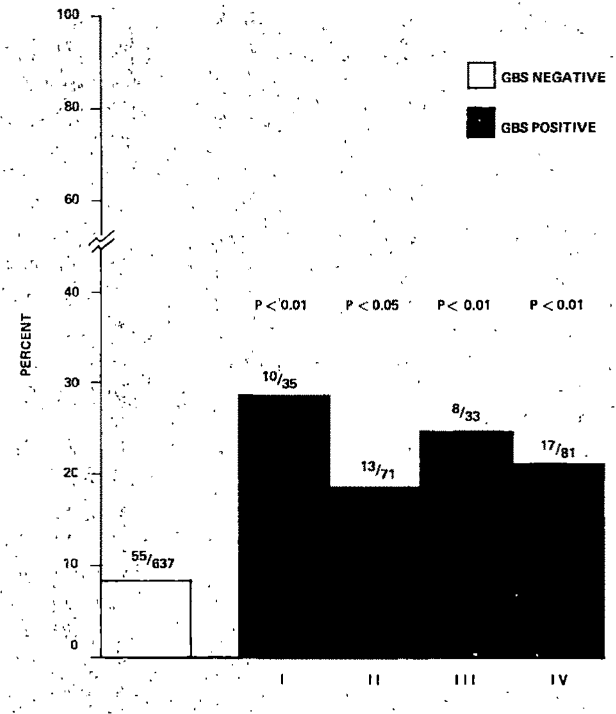

Fig. 3. Frequency of complicated pregnancy in group B streptococcal carriers and noncarriers. *I*: Patients colonized by group B streptococci at 28 weeks; *II*: those having one or more positive prenatal cultures; *III*: those colonized at delivery; *IV*: all women who ever had positive cultures during pregnancy.

groups 3 and 4 (as defined in Fig. 3) when compared with that of patients who had negative cultures throughout pregnancy (Table II). Pelvic infections were also twice as frequent in group 2. This difference approaches significance ($p = 0.06$).

The above data do not reflect the impact of primary cesarean section on the frequency of infection. When this variable was considered, 88% (21 of 24) of pelvic infections were in women delivered by primary cesarean section (Table II). Although infection was more frequent in group B streptococcal carriers delivered by primary cesarean section, the differences were not statistically significant. The number of patients in the carrier group, however, is small. Also the data are incomplete because two infected patients did not have cultures taken at delivery. Furthermore, primary cesarean section was twice as frequent in the carrier group (11%, nine of 81, versus 5%, 32 of 637). For these reasons we were unable to assess satisfactorily the possibility of an added influence of group B streptococci on post–cesarean section morbidity and we believe that question requires further investigation. It does seem clear, however, that group B streptococcal carriers, whether detected during pregnancy or on admission for delivery, are not at increased risk for pelvic infection unless they are delivered by primary cesarean section. No prenatal group B streptococcal carriers or women colonized at delivery had pelvic infections if delivery was by repeat cesarean section or by the vaginal route.

Low birth weight and premature rupture of membranes. There are several reasons for believing a cause-and-effect relationship exists between group B streptococcal carriage and preterm birth. Early group B streptococcal neonatal sepsis is seen more frequently in preterm newborn infants[2]; anecdotal associations are common, and delivery prior to 32 weeks' gestation is increased in group B streptococcal carriers detected by cultures on admission.[3] Although these observations are the basis for considering prenatal group B streptococcal detection and eradication, the magnitude of risk and predictive value of prenatal screening cultures are not known. We found the frequency of low–birth weight deliveries to be increased in prenatal group B streptococcal carriers when compared with noncarriers (Table II, 8% versus 3%, $p = 0.05$), but no differences were seen in these groups at delivery (6% versus 3%, p = NS). Three of the six low–birth weight infants in the prenatal group B streptococcal group weighed more than 2000 gm at birth. Three mothers had negative cultures on admission; two had positive cultures, and one did not have a culture obtained.

A second concern is that group B streptococcus is an etiologic factor in premature rupture of the membranes. This concept, if true, has important implications since premature rupture of the membranes is an event known to precipitate preterm labor and delivery. Although an association has been shown between group B streptococcal carriers on admission and premature rupture of the membranes, the colonized population included both preterm and term pregnancies.[3] Since the primary concern in pregnancies complicated by premature rupture of the membranes is preterm de-

Table II. Comparison of the frequency of perinatal complications in pregnancies with group B streptococcal positive and negative cultures

	Negative (n = 637)		*Positive*								
			Total (n = 81)			*Prenatal (n = 71)*			*Delivery (n = 33)*		
Perinatal complication	*No.*	*%*	*No.*	*%*	*p**	*No.*	*%*	*p**	*No.*	*%*	*p**
Pelvic infection	17	3	7	9	<0.05	5	7	NS	4	12	<0.05
Pelvic infection per number of primary cesarean sections	14/32	44	7/9	78	NS	5/7	71	NS	4/4	100	NS
Delivery of low–birth weight infant	22	3	6	7	NS	6	8	0.05	2	6	NS
Premature rupture of membranes and delivery of low–birth weight infant	11	2	4	5	NS	4	6	0.05	2	6	NS
Suspected neonatal sepsis	22	3	7	9	<0.05	5	7	NS	5	15	<0.01

There were 33 repeat cesarean sections with 27 negative cultures and six positive cultures. There were no pelvic infections.
*Comparison with negative culture group.

livery, it seems more appropriate to consider this group separately. We limited our observations to premature rupture of the membranes associated with low–birth weight deliveries (Table III). Again, as with low–birth weight infants, this complication was more frequent in prenatal group B streptococcal carriers (6% versus 2%, $p = 0.05$) but not in carriers at delivery. Of the four pregnancies in the prenatal group B streptococcal group with premature rupture of the membranes and low–birth weight infants, three infants weighed more than 2000 gm at birth. Two mothers had positive group B streptococcal cultures, one had a negative culture, and one did not have a culture obtained at delivery.

The above differences are undoubtedly affected by spontaneous group B streptococcal loss and acquisition during pregnancy and the fact that multiple cultures were done in the prenatal group. They may also be influenced by risk factors not considered in this study (for instance, parity, previous low–birth weight infant, smoking, multiple gestation). We interpret our data to indicate that group B streptococcal carriers may be at greater risk for premature rupture of the membranes and/or low–birth weight infants than noncarriers. That risk, however, has little clinical significance since 92% of group B streptococcal carriers, either prenatal carriers or carriers at delivery, were delivered of infants weighing >2500 gm.

Neonatal sepsis. The frequency of neonatal sepsis in group B streptococcal carriers and noncarriers is compared in Table II. The diagnosis was made by the pediatric staff and was based on various clinical parameters as there were no culture-proved cases in either group. Prenatal group B streptococcal colonization was not found to be associated with an additional risk for neonatal sepsis. However, this complication was significantly increased in women who had positive cultures at delivery (15% versus 3%, $p < 0.01$). Although preterm labor and preterm premature rupture of the membranes are considered risk factors for group B streptococcal neonatal sepsis,[11] all of the infants in the group with positive cultures at delivery weighed >2500 gm.

Perinatal deaths. There were six perinatal deaths; five were in the group with negative cultures and one was in the group with positive cultures. The single death in the latter was in a 3110 gm male infant delivered by repeat cesarean section following spontaneous onset of labor with intact membranes. The Apgar scores were 7 and 8 but a diagnosis of acute pneumonia was made shortly after delivery. Cultures from the sepsis workup were negative and no autopsy was done. The mother was never febrile and received no antibiotics following operation but had a positive culture for group B streptococci on admission.

Comment

Awareness of group B streptococcal carriage frequently influences clinical management. For example, some physicians treat group B streptococcal carriers with premature rupture of the membranes and those undergoing repeat cesarean section with antibiotics. In other instances, infants delivered of group B streptococcal colonized mothers are selectively evaluated and treated for sepsis. Also, in our hospital, neonatologists favored prenatal detection and antibiotic treatment of group B streptococcal carriers. This practice reflects physician bias concerning the dangers of group B streptococcal carriage. Awareness of the fulminant nature of early group B streptococcal sepsis and fear of that complication are understandable concerns. It seemed unethical, therefore, to report culture results but re-strict physicians from acting in accordance with their professional judgment. A factual basis for medical management, however, is an equally legitimate concern. Since both of these views have merit and because a consensus standard of practice does not prevail, we convinced our human use committee, with significant opposition, to approve a protocol which blinded the culture results.

We are concerned about the confusion and bias that have evolved in the past decade in regard to the risk, prevention, and management of morbidity related to group B streptococci and believe these conditions exist because the data base is incomplete and the problem poorly defined. We know that group B streptococci cause neonatal sepsis, maternal infection, and possibly some preterm births. It is equally clear that other microorganisms are responsible for similar problems. What the obstetrician needs to know is whether risks are greater for prenatal group B streptococcal carriers. If so, is the magnitude of risk sufficient to justify prenatal prevention and are such measures effective?

The medical and scientific need to clarify the above issues is obvious but there is another concern. We live in a litigious society and the obstetrician appears to be an increasingly frequent target. When the outcome is bad, facts unclear, and biased voices argue for unproved schemes, the risk of litigation is increased. For all of these reasons an authoritative, consensus opinion needs to be developed concerning the risk of group B streptococcal morbidity and the efficacy and cost-effectiveness of prenatal prevention.

In this study a point of reference was established concerning the frequency of complicated pregnancies in group B streptococcal carriers (Fig. 3). Up to 28% of such pregnancies were associated with morbidity. This represents a threefold increase over that in noncarriers and is a rather impressive difference. If our perspective were limited to this collective assessment of morbidity, prenatal group B streptococcal detection and eradication would seem reasonable. When the complications are considered individually, however, the impact of such programs is less convincing.

We found the frequency of pelvic infection to be greater in group B streptococcal carriers than in noncarriers (9% versus 3%, Table II) and this complication accounted for 29% (seven of 24) of total group B streptococcal morbidity. Nevertheless, we do not consider prenatal preventive measures to be indicated for this problem because infection was limited to women delivered by primary cesarean section. Since infection did not occur in group B streptococcal carriers delivered by repeat cesarean section or by the vaginal route, these women would not benefit from prenatal prophylaxis. Furthermore, most obstetric services, including ours, currently use some perioperative antibiotic prophylaxis regimen because of the high infection risk associated with primary cesarean section. Although group B streptococcal colonization may pose an additional risk for these patients, there is no evidence to suggest they are not protected by current prevention methods.

Group B streptococcal colonization may be an etiologic factor in some preterm births. This is the most compelling reason to consider prenatal prevention. An association was first suggested by early reports which noted that neonatal group B streptococcal sepsis was more common in low–birth weight infants.[10] More recently Regan et al.[3] reported more tangible evidence of a causal role for group B streptococci. They found preterm births (<32 weeks) to be more frequent in parturient patients with group B streptococci than in the total population (5.4% versus 1.8% respectively). Although we compared low–birth weight delivery rather than birth prior to 32 weeks, our findings (Table II), even in prenatal carriers, are similar (8% versus 3%, $p = 0.05$). Our views concerning the clinical significance of these data, however, differ. Ninety-two percent of prenatal carriers and 94% of women colonized at delivery were not delivered of low–birth weight infants. The predictive value of a positive prenatal screening culture was only 8%. Regardless of the issue of efficacy, these findings suggest the potential impact of group B streptococcal carriage on preterm delivery is too small for prenatal prevention to be cost-effective.

There is some confusion regarding the triad of group B streptococcal carriage, premature rupture of the membranes, and preterm delivery. Contrary to common belief, group B streptococcal prenatal carriage has not been associated with premature rupture of the membranes in preterm births. Although Regan et al.[3] did find the incidence of premature rupture of the membranes to be increased in their total population with group B streptococci detected on admission, they did not report the frequency of this complication in their preterm population. We found births complicated by both premature rupture of the membranes and low birth weight to be increased threefold in women colonized during pregnancy and at delivery (6% versus 2%). However, we believe it is more important to address the clinical relevance of these data rather than focus on the statistical significance. If the presence of group B streptococci in the genital tract of the pregnant woman increases the likelihood of preterm premature rupture of the membranes, that risk appears to be small. In our obstetric population 94% of group B streptococcal carriers did not have that complication. Furthermore, the predictive value of a positive prenatal culture in carriers with serial cultures rather than a single culture was only 6%. When viewed from this perspective, it is doubtful that the efficacy of preventing premature rupture of the membranes caused by group B streptococci can be demonstrated.

A confirmed diagnosis of neonatal sepsis is based on positive cultures of body fluids. The attack rate for neonatal group B streptococcal sepsis, based on that definition, is 3 to 4.2 per 1000 live births.[2] Since none of the infants evaluated and treated for infection in this study met this criterion, the significance of our observations concerning this complication are a matter of conjecture. We cannot explain the significant difference in the frequency of neonatal sepsis at delivery (15% versus 3%, $p < 0.01$). Although the risk of sepsis has been found to be increased in group B streptococcal carriers admitted in premature labor and with preterm premature rupture of the membranes,[11] none of the infants treated for sepsis in the group with group B streptococci at delivery weighed <2500 gm. Ninety-three percent of infants delivered of prenatal group B streptococcal carriers did not develop signs and symptoms suggestive of sepsis.

In summary, complicated pregnancy was at least twice as frequent in prenatal group B streptococcal carriers as in noncarriers. Maternal pelvic infection, which accounted for approximately 30% of the morbidity in colonized women, occurred only in patients delivered by primary cesarean section, a group now managed with perioperative antibiotic prophylaxis. Clinical neonatal sepsis accounted for another 30% but prenatal screening was not predictive of that complication. The remainder were associated with premature rupture of the membranes and/or low–birth weight delivery. Since ninety-two percent of prenatal group B streptococcal carriers were not at risk for delivery of low–birth weight infants it seems unlikely that obtaining cultures and treating women during pregnancy would be cost-effective. We do not believe routine prenatal screening for group B streptococci is indicated until prospective studies demonstrate the value of such programs.

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Long-term neurological morbidity in breech and vertex births

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Seventy frank breech fetuses delivered vaginally, alive at the onset of labor, and without major congenital anomalies were matched by birth weight and race with a breech infant born by cesarean section, a vertex infant born vaginally, and a vertex infant born by cesarean section. Long-term neurological abnormality was determined from time of hospital discharge through a minimum of 2 years of age. The results document the absence of significant differences with respect to major brain damage effected by birth route changes for breech fetuses and between the entire breech and vertex populations. (AM J OBSTET GYNECOL 1985;151:718-20.)

Key words: Breech, neurological outcome, fetus

During the past 20 years, significant changes have taken place in the management of labor. As seen in individual reports[1] and the massive review of the Consensus Developmental Conference on Cesarean Births,[2] the cesarean section has become a major obstetric intervention used to overcome problems of labor and delivery and to avoid increased risk to mother and fetus. Specifically, the use of forceps and more accurately midforceps has diminished, as has the increased use of the cesarean section for fetal distress or dystocia and the repeat cesarean section.[2] In addition, the cesarean birth route has become the more common delivery mode in breech birth.[2] Most recently, suggestions have been made by some to consider the very low–birth weight fetus, by virtue of its birth weight alone, as possibly being benefited by the cesarean birth route.[3]

In an earlier report describing the patient population at Cleveland Metropolitan General Hospital between 1976 and 1982, the effect of birth route on mortality and neonatal morbidity in the frank breech presentation demonstrated that, when stratified by birth weight, neither neonatal mortality nor morbidity was demonstrably affected by change in birth route.[4] A caveat in the breech study was that the evaluation of long-term infant neurological morbidity was not available.

In a parallel report on vertex births,[5] similarly stratified by birth weight, no significant improvement in neonatal mortality or morbidity was associated with the cesarean birth route. In addition the trend was observed for increase in risk in the cesarean birth group at the non–low birth weight (>2500 gm) categories. It was anticipated this increase in risk may reflect reasons for choice of cesarean section such as fetal distress. Once again, long-term morbidity (following hospital discharge) was not studied.

In those two studies of both frank breech and vertex births, confounding variables such as major congenital anomalies and prelabor stillbirths (as demonstrated by a recorded heartbeat during labor) were eliminated because of their obvious associations with birth route choices. Our concern in those reports was that <4.0% of the outcome variance for neonatal neurological damage could be explained prior to discharge.

In this report, long-term (2 years or longer) infant neurological morbidity was selected as the dependent variable. Our hypothesis was that in the frank breech presentation the vaginal birth route did not present an increased risk for neurological damage when compared with the cesarean route.

Methods

From the original study population, frank breech vaginal births were identified of infants who were alive at discharge from the hospital and for whom record reviews could be obtained for at least 2 years of follow-up. Major brain damage was identified as either morphologically identified changes such as hydrocephalus or microcephaly or functional neurological abnormality such as mental retardation or cerebral palsy. The diagnosis was confirmed by chart review as assessed by one of the authors (R. M. B.) without knowledge of presentation or birth route.

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Table I. Summary description of characteristics of matched groups.

Sample	*Breech-vaginal*	*Breech–cesarean section*	*Vertex-vaginal*	*Vertex–cesarean section*
n	70	70	70	70
Average weight	2643.32	2645.53	2657.21	2666.07
SD	365.82	837.57	841.46	846.63
White (%)	71	71	71	71
Male (%)	58	46	49	43
Children with abnormalities				
No.	6	4	1	7
%	9	6	1	10

Table II. Statistical significance of differences in proportion of abnormalities between breech and vertex presentations and between vaginal and cesarean birth routes by type of presentation

Group	*Test statistic*	*Degrees of freedom*	*Significance*
Breech (vaginal and cesarean section) versus vertex (vaginal and cesarean section	$Q_{diff} = 0.25$	1	NS
Breech-vaginal versus breech–cesarean section	$Q_{breech} = 0.50$	1	NS
Vertex-vaginal versus vertex–cesarean section	$Q_{vertex} = 4.50$	1	$p < 0.05$
Total	$Q = 5.25$	3	

To accomplish the stated goals a matching strategy, performed with computer assistance, was developed which prospectively selected 70 quadruples of subjects matched by race and birth weight under the restriction that one of the subjects had a breech presentation and had been delivered vaginally, another had a breech presentation and had been delivered by cesarean section, a third had a vertex presentation and had been delivered vaginally, and a fourth had a vertex presentation and been delivered by cesarean section. The proportions of subjects with abnormalities from the four resulting matched samples were then compared.

To avoid the pitfalls of multiple comparisons, in the statistical analysis the general method for partitioning a χ^2 statistic described by Everitt[6] was used. This method uses advantageously the experimental design and, for the proper partition, yields reliable significance probabilities for the comparisons deemed relevant.

In order to accomplish this, the Q statistic, as reported by Cochran,[7] used to test the significance of the overall differences among the four groups was calculated and then partitioned into three key components, Q_{diff}, Q_{breech}, and Q_{vertex}. Q_{diff} compares the proportions of abnormalities in the breech and vertex presentations and thus consolidates the data into two categories, whereas Q_{breech} and Q_{vertex} compare the proportions of abnormalities within the breech presentations (that is, breech-vaginal versus breech–cesarean section) and within the vertex presentations (that is, vertex-vaginal versus vertex–cesarean section), respectively. The Q statistic has in this case three degrees of freedom and may be represented as $Q = Q_{diff} + Q_{breech} + Q_{vertex}$, where each summand has one degree of freedom.

Results

A profile of the group is presented in Table I. The match characteristics for birth weight, sex, and color eliminate these population characteristics as possible confounding variables.

The statistic results are presented in Table II. It is seen that no significant differences between the breech and vertex presentations or between the vaginal and cesarean routes in the breech presentations were detected. There was a significant difference in the proportion of abnormalities between the vaginal and cesarean routes in the vertex population. This will be expanded upon in the Comment section.

Comment

In the original data sets for both breech and vertex studies, when subjects were matched by birth weight, the majority of outcome variance for death and neonatal morbidity was related to both birth weight and gestational age.[4, 5] However, the known outcome variance for neonatal morbidity (prior to hospital discharge) was <4% for both the breech and vertex studies, in contrast to the 35% known outcome variance for breech and almost 17% known outcome variance for vertex with respect to neonatal mortality. It was apparent that neurological morbidity was not easily diagnosed for most neonates prior to hospital discharge.

In the present study a chart review was used to document functional brain deficit. Almost 80% of the original vaginal breech population could be followed up until a diagnosis was made of developmental lag or for a minimum of 2 years. It was felt that although minimal brain damage such as learning disabilities or mild mental retardation was of concern its relationship to socioeconomic factors could not be easily controlled for in this study and its determination from a chart review would be unsatisfactory. In contrast, severe brain damage is less overtly influenced by socioeconomic variables and because of its clinical magnitude is more easily confirmed in chart review.

The vaginal frank breech birth was approximately one third of our laboring frank breech population. When matched by birth weight (within 100 gm in almost all cases) and race, there was no improved outcome conferred by the cesarean birth route choice over the vaginal birth route choice.

It is noted that breech and vertex vaginal births also have a very different experience in terms of births with abnormalities (9% versus 1%). The statistical significance of that difference was not tested in order to avoid the problems of multiple comparisons testing. Thus the experimental design did not allow one to place a probability that such a difference might have occurred by chance.

In contrast to the breech birth route comparisons, the vertex cesarean birth group was at greatest risk and statistically different from the vaginal vertex group. This suggests that the reasons for the cesarean section which added risk beyond birth weight and maturity, such as fetal distress, contributed to the observed morbidity.

In completing this third study on the same data set, it is evident that the change to cesarean birth for the fetus in frank breech presentation in labor requires careful review. Alteration in birth route does not appear to improve outcome with respect to mortality and morbidity. A fetus in frank breech presentation, without major congenital anomalies and alive at the onset of labor (the study criteria), does not have increased risk when compared with a similar group of vertex births. It is also apparent that the studies of the problem of breech presentation and birth route are often confounded by the high incidence of low birth weight and congenital anomalies, two variables which were controlled for in this report and which are not altered by birth route choices.

A similar statement relating to outcome may be made for the vertex and breech populations delivered vaginally when compared with breech and vertex infants delivered by cesarean section. As noted in the earlier two studies[4, 5] and in the present study, altering birth route by choice for cesarean section does not appear to alter outcome. Additional factors (such as fetal distress) may contribute to increased risk and are more evident in the vertex population born by cesarean section.

Finally one may state that there is a need for prospective and randomized studies for both breech and vertex fetuses at the very low birth weights (<1500 gm) if the trend to increased use of the cesarean birth route to improve infant outcome is to be justified.

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Brain injury and intrauterine death

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Many of the stresses contributing to brain injury in the intrapartum or neonatal period may also exist prenatally. To define this problem, we reviewed the clinical features and neuropathologic findings in 433 consecutive stillbirth autopsies. Twenty-five had evidence of periventricular/intraventricular hemorrhage or gliosis. Twenty infants had died in utero before active labor and five during delivery. Nine stillbirths were infected. Ten cases had periventricular/intraventricular hemorrhage alone, five had the hemorrhage with parenchymal hemorrhage, five had parenchymal hemorrhage only, and five had gliosis. This group of 25 cases establishes that intrauterine brain injury is not rare. Separation of antepartum events occurring in utero from those imposed during labor, delivery, resuscitation, and the neonatal period is very important and has significant medical and legal implications. (AM J OBSTET GYNECOL 1985;151:721-3.)

Key words: Fetal death, brain lesions

It is well established that various forms of stress during delivery and the neonatal period may impair cerebral perfusion, cause brain injury, and subsequently lead to neurologic impairment. It is likely that many of the factors that compromise cerebral blood flow in a neonate also exist in the fetus. The recognition of brain damage in the fetus is diagnostically difficult and little is known of its occurrence. In an effort to better define the problem, we reviewed the clinical features and neuropathologic findings of our stillborn population by using central nervous system hemorrhage and gliosis as markers for fetal brain injury. Recognition of fetal brain injury may provide answers to unexplained intrauterine deaths and neurodevelopmental impairment in survivors. It may also address the related medicolegal issues surrounding such cases.

Material and methods

The autopsy records of the Los Angeles County–University of Southern California Medical Center Women's Hospital from January 1, 1978, through December 31, 1983, were surveyed for cases of intrauterine injury to the central nervous system. The same routine autopsy procedure was followed in each case. The brains and spinal cords were removed, fixed in 10% phosphate–buffered formalin and examined after a minimum of 3 weeks' fixation. The brains were coronally sectioned, and selected areas of cerebrum, cerebellum, brain stem, and spinal cord were taken for histologic examination. The neuropathologic data were retrospectively reviewed for hemorrhage, which serves as a marker for significant blood flow disturbance in the central nervous system, and gliosis, which may indicate chronic hypoxic damage.

During the 6 years of the study, 433 autopsies were performed on stillborn infants. A total of 72 were found to have evidence of central nervous system injury, an incidence of 17%. Cases were excluded if there was only subarachnoid, subdural, intradural, meningeal, or choroid hemorrhage, any associated central nervous system anomalies, or severe maceration precluding adequate evaluation. Forty-seven cases were excluded, leaving a study population of 25 with periventricular/intraventricular hemorrhage, parenchymal hemorrhage, or gliosis at autopsy. The history of the pregnancy and delivery of these 25 cases was reviewed from the mother's charts.

Results

Twenty of the 25 infants in the study died in utero before the onset of active labor, but only 12 of the mothers reported decreased or absent fetal movements for a minimum of 24 hours before delivery. Five of the fetuses died during the intrapartum period. Pregnancy was complicated in 21 cases (Table I). Clinical amnionitis was diagnosed in nine of the mothers and this was correlated with evidence of infection in seven of the infants. Two infants without a history of maternal infection were also found upon postmortem examination to be infected. Pneumonia was found in four autopsies, positive blood culture in seven, and funisitis in three

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Table I. Characteristics of the patients

Birth weight (gm)	*Gestational age (wk)*	*Pertinent findings*	*Stillbirth infection*	*Central nervous system pathology*
820	25	Vaginal bleeding, positive VDRL, amnionitis	Positive blood culture	Cerebral gliosis
840	27	Hydrops fetalis	None	Cerebral hemorrhage
840*	26	Amnionitis	Positive blood culture, pneumonia	Periventricular/intraventricular hemorrhage, brain stem hemorrhage
895	27	Amnionitis	Funisitis	Periventricular/intraventricular hemorrhage
1160	30	Amnionitis	Pneumonia, funisitis	Periventricular/intraventricular hemorrhage
1240	30	Prolapsed cord, teenage mother	None	Cerebral hemorrhage
1580	34	Lupus erythematosis, vaginal bleeding	None	Periventricular/intraventricular hemorrhage
1830*	30	Amnionitis	Positive blood culture, pneumonia, funisitis	Periventricular/intraventricular hemorrhage
2200*	35	Unremarkable	Positive blood culture pneumonia	Periventricular/intraventricular hemorrhage, cerebral hemorrhage
2210	38	Amnionitis	None	Cerebral artery emboli, periventricular/intraventricular hemorrhage, cerebral and cerebellar hemorrhage
2500	37	Abruptio placentae, pregnancy-induced hypertension	None	Cerebral hemorrhage
2500	35	Nuchal cord	None	Periventricular/intraventricular hemorrhage
2700*	41	Amnionitis, meconium-stained fluid	None	Periventricular/intraventricular hemorrhage
2800	38	Amnionitis	Positive blood culture	Periventricular/intraventricular hemorrhage
2950	39	Twin	None	Periventricular/intraventricular hemorrhage
3080	40	Unremarkable	None	Cerebral gliosis
3100	34	Pregnancy-induced hypertension	None	Cerebral gliosis
3320	46	Malaria	None	Cerebral gliosis
3460	41	Amnionitis, pregnancy-induced hypertension	Positive blood culture	Cerebrum and cerebellum gliosis
3500	40	Vaginal bleeding	None	Periventricular/intraventricular hemorrhage with fibrosis, cerebellar hemorrhage
3530	40	Vaginal bleeding	Positive blood culture	Cerebellar hemorrhage
3600	42	Teenage mother, meconium-stained fluid	None	Periventricular/intraventricular hemorrhage
3980*	40	Gestational diabetes, pregnancy-induced hypertension	None	Periventricular/intraventricular hemorrhage, cerebral hemorrhage
3945	40	Unremarkable	None	Cerebral hemorrhage
4000	40	Meconium-stained fluid	None	Periventricular/intraventricular hemorrhage

*Intrapartum death.

(Table I). Third-trimester bleeding was reported in four mothers. The mean maternal age (±SD) was 25 ±6 years.

Nine of the infants were female and 16 were male. The mean birth weight (±SD) was 2443 ±1063 grams, and the mean gestational age was 36 ±5 weeks. Three infants were meconium stained at birth. One infant was hydropic secondary to hemolytic disease of the newborn. Vascular anomalies were not found in any of the cases, although cerebral artery emboli were found in one small–for–gestational age infant with a maternal history of amnionitis.

Periventricular/intraventricular hemorrhage was the most common form of hemorrhage and it occurred in 15 cases. In 10 patients the hemorrhage was limited to the ventricles, and in five the cerebrum, cerebellum, and/or brain stem were involved as well. The most extensive hemorrhage occurred in the infant with cerebral artery emboli who had periventricular/intraventricular hemorrhage as well as cerebral and cerebellar hemorrhage. One cerebellar and four cerebral hemorrhages occurred without periventricular/intraventricular hemorrhage. All of the hemorrhages appeared acute except one in which intraventricular fibrosis was found.

Gliosis was found in five cases, four involving the white matter of the cerebrum and one involving both the cerebrum and cerebellum (Table I). Central nervous system infection was not found in any of the cases, but two cases with gliosis had a positive prenatal history for infection. One mother had a positive VDRL test and amnionitis, and her infant had a positive postmortem blood culture. The other infant, who was 46 weeks by dates, did not have evidence of infection, but his mother was treated for malaria with quinine during the first trimester.

Comment

Several case reports of the ultrasound diagnosis of fetal intracranial hemorrhage have appeared in the radiologic literature, the first by Kim and Elyaderani in 1982.[1] In four of the reported cases no known precipitating events or underlying fetal disease was identified.[1-4] In one case maternal isoimmune thrombocytopenia was present[5] and in another a rapid onset of preeclampsia with an associated abruptio placenta occurred.[6] The association with amnionitis we found in many of our cases has not been described. Thirty-six percent of the cases in this series of intrauterine brain injury had evidence of acute intrauterine infection compared to an overall rate of 20% in our stillborn autopsy population.[7] Presumably the infection leads to cardiovascular compromise and concomitant alterations in cerebral blood flow. It is interesting that in one of the cases with maternal amnionitis, cerebral artery emboli were found. These emboli could have originated in association with placental vasculitis,[8] but unfortunately the placenta was not examined in this case.

The pattern and distribution of the central nervous system hemorrhages found in this study are similar to those commonly found postnatally. These hemorrhages in the neonate are currently thought to result from impaired regulation of cerebral perfusion and hypoxia,[9] and such insults may also occur antenatally.[10]

It is difficult to explain the gliosis we found in five of the cases in this study. All five infants were known to have died in utero before the onset of labor. Gliosis is a marker of previous injury including chronic hypoxia with scarring, but it was not possible to determine the underlying cause in these cases. Previous central nervous system infection or ischemic damage could account for the change, but neither could be documented with certainty in our cases.

This group of 25 cases establishes that intrauterine central nervous system injury is not rare. Undoubtedly many fetuses with less compromise but similar lesions may be live born, and they would have decreased tolerances for labor and delivery. Some infants born with similar central nervous system injuries may be left with unexplained neurologic handicaps. Separation of adverse antepartum events in utero from those imposed during labor, delivery, resuscitation, and the neonatal period is very important. Documentation of antepartum brain injury will help clarify complex medical and legal questions.

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The role of external version in the intrapartum management of the transverse lie presentation

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External version under tocolysis was applied and studied prospectively as an alternative to routine cesarean delivery in the laboring patient with a transverse lie presentation. After sonographic confirmation and signing of informed consent, 12 patients with a transverse lie presentation and intact membranes agreed to participate in the current investigation. For these 12 patients, 10 (83%) presentations were successfully converted to a longitudinal lie (nine cephalic, one breech) and two (17%) remained transverse. Of the 10 patients with successful conversions, six (60%) delivered vaginally while four required cesarean delivery. In two (20%) patients external version was unsuccessful and both patients were delivered by cesarean. With the exception of one infant with congenital anomalies, neonatal outcome was excellent in the study population. Overall, the use of external version in the laboring patient with a transverse lie presentation was associated with a 50% reduction in the cesarean section rate. While these results are encouraging, further experience is still needed to more clearly define the maternal and fetal risks associated with this procedure. (AM J OBSTET GYNECOL 1985;151:724-6.)

Key words: Transverse lie, external version

External version under tocolysis has been shown to be an effective technique for the antepartum management of the term breech presentation.[1-4] Based on these observations in the breech presentation, external version was considered as an alternative to routine cesarean section in the patient with a transverse lie presentation in labor. Traditionally, the term fetus in a transverse lie in labor is delivered by cesarean section, frequently through a vertical uterine incision. As an alternative, external version provides an attempt to achieve a longitudinal lie, which may (1) allow greater opportunity for vaginal delivery and (2) permit safe utilization of the low transverse uterine incision for a cesarean birth.

The purpose of this report is to describe an experience with external version in laboring patients with a transverse lie presentation.

Material and methods

Patients who presented in labor with a transverse lie presentation, an estimated fetal weight ≥2000 gm or ≥34 weeks' gestation, and intact membranes were considered for inclusion in the current study. Additional entry criteria for an attempted external version included a normal ultrasound evaluation and either a reactive nonstress test or a negative contraction stress test. A normal ultrasound evaluation was defined as follows: (1) singleton fetus in a transverse lie position, (2) adequate amniotic fluid (≥5 cm of total fluid over four quadrants), (3) absence of any gross fetal anomaly, and (4) absence of placenta previa.

Patients with the following conditions at the time of evaluation were excluded: (1) ruptured membranes, (2) placenta previa, (3) oligohydramnios (<5 cm total over four-quadrant evaluation), (4) multiple gestation, (5) evidence of uteroplacental insufficiency, (6) contraindication to β_2-mimetics, (7) amnionitis, (8) third-trimester bleeding, or (9) previous uterine scar. After confirmation of the transverse lie and a patient's candidacy for attempted version, informed consent was obtained.

All procedures were conducted in the labor and delivery suite. The patients were placed in the supine position with left lateral displacement of the uterus. An intravenous line was established with lactated Ringer's solution. Pelvic examination was performed. Ritodrine (Yutopar, Astra Pharmaceutical Products, Worcester, Massachusetts) was infused at a constant rate of 100 μg/min for 10 to 15 minutes prior to and during the version attempt. Maternal heart rate and blood pressure were recorded every 5 to 10 minutes. Fetal heart activity was evaluated during the version with either real-time B-scan or electronic monitoring (Doppler ultrasound). After the version, the fetal heart rate was continuously monitored.

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If uterine activity was persistent after about 10 to 15 minutes of ritodrine infusion at 100 μg/min or irritability was found with uterine manipulation, the dosage was serially increased by 50 μg every 10 minutes to a maximum dose of 350 μg/min to try to ablate uterine contractility. If maternal or fetal side effects were excessive, the dosage was decreased.

Version was accomplished by either the classic forward roll or the newer back-flip of Saling and Muller-Holve.[2] With a back-up transverse lie, a forward roll was attempted first; in the back-down transverse lie, a back-flip was initially attempted. If the first technique was unsuccessful, the opposite method was tried.

The procedure was interrupted if discomfort became intolerable for the patient or if fetal heart rate decelerations were noted. If the fetal heart rate decelerations resolved quickly, further manipulation was undertaken. Once the fetus was turned, the tocolytic was discontinued. The fetus was maintained in the new attitude for several minutes until uterine tone was increased and real-time B-scanning confirmed the version outcome. If the cervix was adequately dilated and the patient had been converted to a longitudinal lie, the membranes were ruptured and a fetal electrode was applied and direct fetal heart rate monitoring begun.

Outcome was measured in the following manner: (1) success or failure to convert the fetus to a longitudinal lie, (2) mode of delivery, (3) birth weight, (4) Apgar scores, (5) meconium at delivery, (6) abnormal cord position, (7) maternal and neonatal complications, and (8) pertinent findings with regard to uterine and/or neonatal anomalies.

Results

Between February 20, 1983, and February 19, 1984, 16,674 patients were delivered at the Los Angeles County–University of Southern California Medical Center; 69 (0.4%) presented with a transverse lie and in labor. Of the 69 patients with a transverse lie, 42 were excluded for the following reasons: prior cesarean birth, 17; rupture of membranes, 7; estimated weight <2000, 5; placenta previa, 3; other causes, 10. No patients were excluded because of reduced amniotic fluid volume. In the remaining 27 patients, 14 underwent primary cesarean delivery without consideration for inclusion in the study. Of the 13 patients who were considered for the current investigation, 12 accepted and underwent attempted version (Table I). One patient refused attempted version and underwent a primary cesarean section. As noted in Table I, the 12 patients who agreed to attempted version were at term, and 10 (83%) had had one or more term pregnancies.

At the time of external version, the patients had an average cervical dilatation of 3 cm (Table II). External version was successful in achieving a longitudinal lie in 10 (83%) patients. Similar success rates were obtained irrespective of the position of the fetal back. Version was successful in both of the nulliparous patients. Of those successfully converted to a longitudinal lie, nine were converted to a vertex and one to a breech presentation.

Table I. Demographic features of patients with transverse lie presentations in labor who underwent attempted external version (n = 12)

	Mean	*Range*
Age (yr)	31.2 + 7.2	20-43
Gravidity (n)	4.8 + 3.2	2-12
Parity (n)	2.7 + 2.5	0-9
Gestational age (wk)	38.9 + 2.5	33-42
Height (in)	60.3 + 1.3	58-62
Weight (lb)	150 + 25	116-197
Amniotic fluid volume (cm)	15.4 + 8.3	5-34

Table II. Pregnancy outcome for those patients with transverse lie presentations who underwent attempted external version during labor

	Fetal back		*Overall*	
	Down	*Up*	*n*	*%*
Attempted (no)	5	7	12	
Mean cervical dilatation (cm)	3	2	3	
Version successful (n)*	4	6	10	83
Meconium (n)	2	1	3	25
Cesarean birth (n)	3	3	6	50
Apgar score <7 (n)				
1-min	2	3	5	42
5-min	0	1	1	8

*Longitudinal lie.

In the successful version group, six of the 10 subsequently were delivered vaginally. Four patients underwent cesarean delivery. The indications for cesarean delivery were arrest of dilatation (2), breech presentation (1), and arrest of descent (1). In the first patient a cesarean delivery was performed for arrest of dilatation. This patient delivered a 4530 gm infant with Apgar scores of 4 and 8 at 1 and 5 minutes, respectively. The second patient underwent a cesarean delivery for arrest of dilatation. A 3440 gm infant with meningomyelocele and Apgar scores of 1 and 4 at 1 and 5 minutes was delivered. At birth the infant sustained a spontaneous pneumothorax and was resuscitated. Subsequently the infant has done well at 3 months of age. The patient converted to breech presentation had previously been delivered of a 4400 gm infant in vertex position without difficulty and had an estimated fetal weight of 4500 gm. In view of that obstetric history, an external version was attempted. She was converted to a breech presentation; however, because of macrosomia and breech presentation, a cesarean delivery was subsequently performed. At birth, the infant weighed 4720 gm and had Apgar scores of 6 and 9 at 1 and 5 minutes, respectively. The last patient was a 37-year-old woman, gravida 5, para 2-0-2-2, who was delivered by cesarean section of a 2350 gm infant with craniosynostosis, a 26 cm umbilical cord, and Apgar scores of 6 and 7 at 1 and 5 minutes.

No serious maternal or fetal complications in the current study were noted. Meconium was observed in three instances (trace in labor in one and at delivery in two). One baby had an abnormal cord position noted at delivery. This umbilical cord had looped around the leg of the baby and measured 26 cm. Two infants were anomalous. These infants had craniosynostosis and meningomyelocele. At delivery, none of the study patients were found to have a uterine anomaly.

Comments

Transverse lie presentation in labor is a relatively uncommon intrapartum event. The incidence has been reported to be in the range of 0.3% to 0.4%.[5] Some proposed reasons for transverse lie presentations in labor include abdominal wall relaxation, high parity, prematurity, pelvic contracture, and placenta previa.[5] In the current report, the most common predisposing factor in our population was high parity. Traditional management has been to deliver such patients by cesarean section.

As an alternative to this approach, external version was attempted and found to be successful in 83% of the study population. The immediate net effect of this approach was a 50% reduction in cesarean delivery and a reduction in maternal and fetal morbidity associated with this process. Last but likewise important, successful version to a longitudinal lie reduces the necessity of a vertical uterine incision even though cesarean section was required, and thus such patients might be candidates for a trial of labor in a subsequent pregnancy.[6]

In summary, external version of the transverse lie presentation during labor appears to be a reasonable alternative to routine cesarean delivery. The importance of proper patient selection, continuous surveillance of the mother and fetus during and after the version, and the capability of immediate cesarean delivery cannot be overemphasized. If the patient is in advanced labor (≥7 cm) with intact membranes and meets the previously mentioned entry criteria, consideration should be given to attempting the procedure in the cesarean section delivery room. As greater experience with this approach is gained, its overall application and safety will be more clearly defined. This approach, as in the case of breech presentation,[4] should contribute to a further modulation of the cesarean section rate.

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The effect of exercise on uterine activity in the last eight weeks of pregnancy

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In order to determine if moderate maternal exercise increased uterine activity, a prospective study was done during the last 8 weeks of pregnancy in 17 women. Two forms of exercise were chosen, weight-bearing (running) and non–weight-bearing (stationary bicycle), to study this hypothesis. The results show that with these types of exercise no increase in uterine activity was noted. This is useful information to convey to pregnant patients ready to engage in a physical fitness program. (AM J OBSTET GYNECOL 1985;151:727-30.)

Key words: Uterine activity, exercise, pregnancy

The issue of exercise and reproduction has received new attention with the recent enthusiasm in physical fitness. A recent report by Artal et al.[1] suggested that exercise could trigger premature labor in certain subjects at risk and indicated that some of their patients reported increased uterine activity when they exercised. Clapp and Dickstein[2] reported, on the basis of a retrospective questionnaire study, that exercise may lead to prematurity and to low birth weight. This increased incidence of prematurity and low birth weight was also noted earlier by Balfour[3] in mothers who worked in a standing as opposed to a sitting position, specifically if work was continued after the fifth month of pregnancy. A recent analysis of the United States Collaborative Perinatal Project (1959 to 1965) also corroborated the fact that there was a reduction in mean birth weight in women working in a standing position versus women working in a sitting position and this reduction was not a reflection of premature delivery.[4] These studies raise important questions, among which are the relation of uterine activity to exercise[5-8] and to type of exercise, standing versus sitting. The purpose of this study was to determine if either weight-bearing or non–weight-bearing exercise at a level which increased maternal heart rate to 70% of the predicted maximum could cause an increase of uterine activity in healthy pregnant women.

Material and methods

Seventeen healthy pregnant women enrolled in a regular exercise program at the YMCA were recruited for this study. All patients gave written informed consent according to a protocol approved by the Committee on Human Research at the Oregon Health Sciences University. The mean age was 31 ± 1 years. Six of seventeen were nulliparous. The average height was 1.65 ± 0.07 m and the average nonpregnant weight was 60 ± 7 kg. All subjects were in their third trimester of pregnancy (32 to 40 weeks) with a mean gestational age of 35 ± 2 weeks. All were receiving prenatal vitamins and one patient received doxylamine-pyridoxine (Bendectin) at the time of the study and another diphenhydramine hydrochloride (Benadryl) 8 hours before the study. Most of the subjects had unusually good health habits and many (95%) of them exercised regularly before becoming pregnant (Fig. 1). The subjects were asked to recall whether they experienced uterine activity in the few days preceding each study. The answer was positive in 18 of 20 studies. All patients were fasting at the time of the study but had eaten, on the average, 2½ hours before the study.

Data were collected at three time periods: an initial 30-minute rest period, an exercise period of 10 to 30 minutes, and a recovery period of 30 minutes. All subjects were studied in an enclosed area (indoor track and field and a laboratory) where temperature and relative humidity remained constant. All subjects lay in a semi-Fowler position during the initial resting period and the recovery. Baseline maternal heart rate by carotid palpation and blood pressure by sphygmomanometer were obtained during rest, immediately after exercise,

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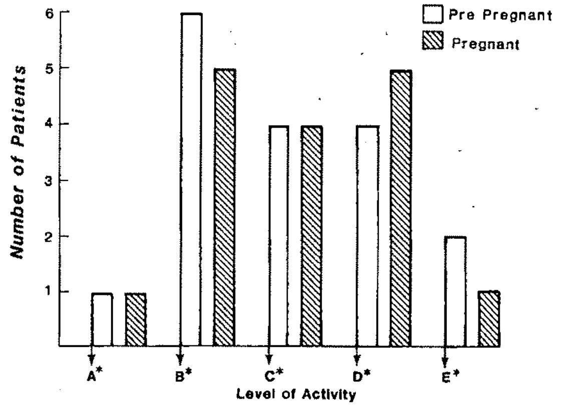

Fig. 1. Level of activity prior to and during pregnancy. *A* = None; *B* = 30 minutes per day; *C* = 30 to 60 minutes per day; *D* = 1 hour per day; *E* = more than 1 hour per day.

and during the recovery period. Fetal heart rate was monitored externally with a Doppler device, and uterine contractions were monitored externally with a pressure tocometer on a two-channel recorder (Hewlett-Packard).

For the exercise phase, subjects were divided randomly into two groups. Ten patients exercised for 30 minutes by walking around a level track (group 1), and 10 patients exercised on a bicycle ergometer between 50 and 60 rpm at an intensity of 50 W for 10 to 15 minutes (group 2). The subjects were asked to alter the speed if walking or to alter the resistance on the ergometer in the attempt to achieve 70% of the individual maximal heart rate as estimated by the subject's age.[9]

Immediately after the exercise all subjects again relaxed in a semi-Fowler position for 30 minutes. Fetal heart rate, uterine activity, and maternal heart rate and blood pressure were measured in a fashion similar to that during the control period. Two tracings of 30 minutes each were obtained during the preexercise and the postexercise periods. In order to eliminate bias during the interpretation of the tracings at a later time, the strips were coded by one of us (K. B.) for each period and decoded only when all of the analysis of the data was completed. These tracings were assessed immediately for heart rate abnormality by one of us (J.-C. V.) and if pathologic findings were noted the patient was to be eliminated from the study. This, however, did not occur. All data were analyzed a few months later in a blinded fashion. Each 30-minute record was divided in half and analyzed as two distinct 15-minute periods in order to assess variability in each of the functions under study and to evaluate the early effect of exercise on uterine activity and fetal heart rate.

Uterine contractions were measured objectively by calculating the area under the curve by a planimeter caliper starting and ending at point *A* after including points *B* and *C* (Fig. 2) (Ott, Dietzzen No. 54097). Fetal heart rates were tabulated at each 20-second mark. An average of 40 fetal heart rate samples were obtained per 15-minute period of the tracing. Data were analyzed by Student's t test for paired or unpaired data. Each patient served as her own control.

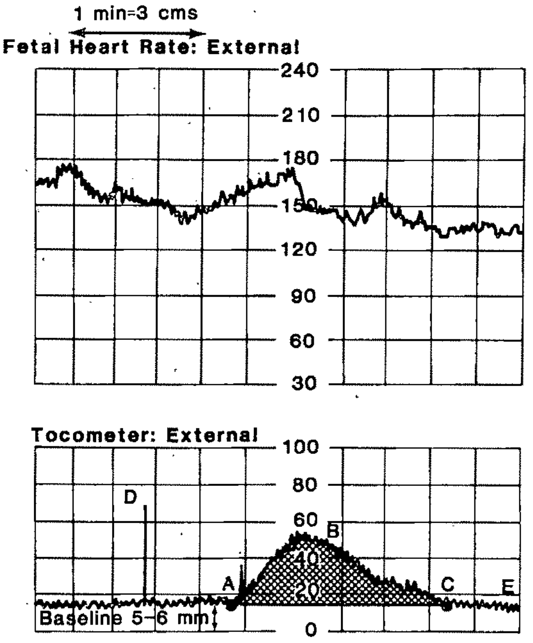

Fig. 2. *Top panel:* Typical fetal heart rate tracing obtained either during rest or in the postexercise period. *Lower panel:* Uterine activity obtained from external tocometer. Belt adjusted to show maternal breathing movements *(E)* and maximum deflection with coughing *(D)*. Planimeter caliper was started at point *A*, included points *B* and *C*, and ended at point *A*.

Results

Maternal mean blood pressure, heart rate, uterine activity, and fetal heart rate in the resting period (preexercise) and immediately after the exercise (postexercise) are shown in Table I. Maternal heart rate significantly increased with exercise ($p < 0.001$). Fetal heart rate was significantly increased during the first 15 minutes of the postexercise recovery period ($p < 0.05$) but returned to baseline in the second 15-minute period. No change in maternal mean blood pressure and mean uterine activity was observed.

The mean gestation at delivery was 40 ± 1 week; 13 of the 17 patients had spontaneous vaginal delivery. Four patients had a cesarean section (one for breech, one for cephalopelvic disproportion, two repeat procedures). The average infant weight was 3802 ± 478 gm and mean Apgar scores were 8 at 1 minute and 9 at 5 minutes. The length of labor averaged 12 ± 6 hours.

When retrospective analysis was done to determine differences between the weight-bearing group (group

Table I. Response to exercise

	Prior to exercise	*After exercise*
Mean blood pressure (mm Hg)	79 ± 7	83 ± 5
Mean heart rate (bpm)	79 ± 9	138 ± 15*†
Mean fetal heart rate (bpm)	141 ± 6	149 ± 9 (1st 15 min)‡ 143 ± 7 (2nd 15 min)
Mean uterine activity (square inches)	0.016 ± 0.05	0.008 ± 0.02

*$p < 0.001$.

†Taken immediately at end of exercise period.

‡$p < 0.05$.

Table II. Response of the subgroups to exercise

Subgroup	*Group A (weight-bearing, n = 10)*	*Group 2 (non–weight-bearing, n = 10)*
Mean blood pressure (mm Hg)		
Before exercise	76 ± 7	83 ± 5*
After exercise	80 ± 3	85 ± 5*
Mean heart rate (bpm)		
Before exercise	77 ± 8	79 ± 10
After exercise	134 ± 10	142 ± 5
Mean fetal heart rate (bpm)		
Before exercise	142 ± 6	140 ± 5
After exercise	151 ± 8	146 ± 11
Mean uterine activity (square inches)		
Before exercise	0.025 ± 0.080	0.006 ± 0.09
After exercise	0.010 ± 0.030	0.006 ± 0.08

*$p < 0.02$.

1) and non–weight-bearing group (group 2), the lengths of gestation at the time of study were different: group 1 = 33 ± 3 weeks versus group 2 = 37 ± 1 week. Resting maternal mean blood pressure, heart rate, uterine activity, and fetal heart rate for each subgroup are shown in Table II. The preexercise and postexercise mean blood pressures were different ($p < 0.02$). No other significant differences were noted.

Comment

Moderate exercise in nonpregnant and pregnant women increases the level of catecholamines.[1, 10] Norepinephrine, however, is released in greater amounts than epinephrine.[1, 10, 11] The uterus has receptors for catecholamines[12] and thus can respond to a sudden increase of these amines; more specifically, intravenous administration of norepinephrine, in term patients, stimulates uterine activity, whereas epinephrine has opposite effects on the uterus.[13] Artal et al.[1] have shown an immediate increase in norepinephrine and epinephrine after exercise and these levels return to normal 15 minutes after exercise. Furthermore, the ratio of norepinephrine to epinephrine at that time is increased, suggesting a greater release of norepinephrine, and thus uterine activity might be expected to be increased.

We did not find an increase in uterine activity in the group that was studied, not even in the first 15 minutes after exercise. This negative result may be due to an inadequate level of exercise. Pulse count appears to be a reliable indicator of the level of exercise.[14] While we did not measure catecholamines, the elevation of heart rate to 70% of maximal seems, however, to be an appropriate stimulus to raise catecholamine levels. The mean maternal heart rate in this study was even higher than that of patients studied by Artal et al.[1]; they found elevated levels of catecholamines immediately after exercise. An alternative explanation of this negative result is that endogenous release of catecholamine in the third trimester does not have a major influence on the nonlaboring uterus because of the lack of uterine receptors for catecholamines or the loss of adrenergic innervation. Huszar and Roberts,[12] however, found that the pregnant term uterus does indeed contain abundant catecholamines and adrenergic innervation and thus is able to respond to the different amines.

Contractions may have occurred only during the exercise period. We measured neither uterine activity nor fetal heart rate during that period for technical reasons. Even though the theoretical possibility of uterine contractions during the exercise period exists, if these were to have an influence on initiating premature labor, the postexercise tracing should have reflected some increase in uterine activity.

The negative result observed may also be due to the inability to pick up uterine activities with external monitoring devices. We took great care to apply the device firmly and adjusted the tension of the strap of the external tocometer so that maternal breathing movements were always visible on the recording. One patient who had mild uterine activity at rest had a flat recording after exercise.

The type of exercise performed by the pregnant patient may have different effects on uterine activity. Exercising in an upright position, with the axis of the uterus pointing caudally, may exert more pressure on the cervix; this in turn may increase uterine activity by a reflex mechanism. Exercising on a bicycle, a non–weight-bearing exercise, may have less gravitational effect, less cervical stimulation, and thus less uterine activity. In this study, however, when the subgroups were analyzed, neither group 1 (weight-bearing group) nor group 2 (non–weight-bearing group) showed an increase in uterine activity.

Thus these data indicate that moderate prenatal exercise in a highly motivated, trained pregnant population does not increase uterine activity in the immediate 30 minutes following the exercise period. Fetal heart rate does not increase during the 30 minutes immediately following moderate exercise. These data help answer objectively the question of the effect of exercise on uterine activity put forward by the ever-increasing pregnant population wishing to indulge in physical fitness activities during gestation.

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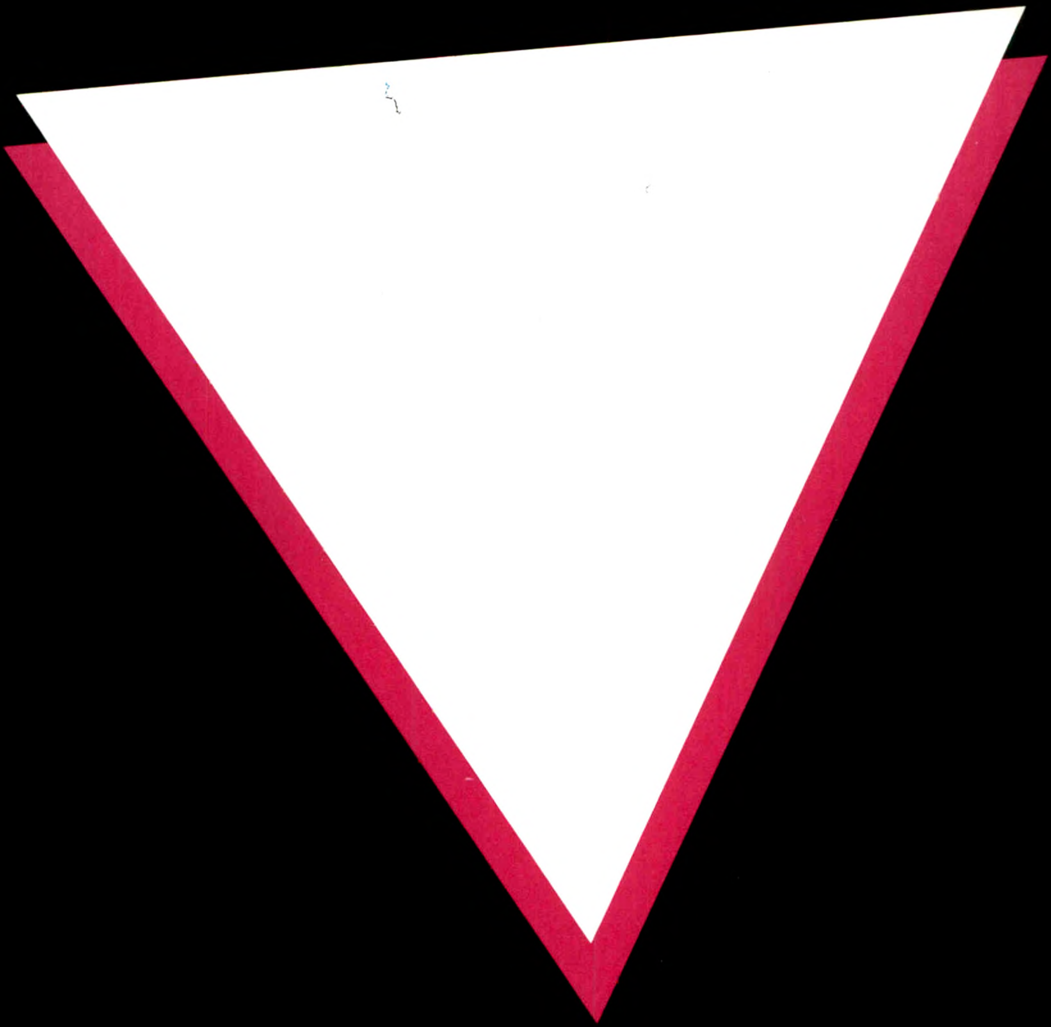

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The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was ap-

Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

Syntex Laboratories, Inc.

Fatal meconium aspiration syndrome occurring despite airway management considered appropriate

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A combined obstetric-pediatric approach to tracheal toilet is said to prevent serious cases of the potentially fatal meconium aspiration syndrome. After delivery of the head a DeLee trap is used to suction the oropharynx and nasopharynx. Immediately following delivery, endotracheal suction is performed in an effort to remove any remaining meconium-stained amniotic fluid. Although routinely using this approach, we continue to have occasional cases of fatal meconium aspiration syndrome. Therefore, we reviewed the outcome of infants born through meconium-stained fluid. During a 5-year period, 1420 (15%) of 9299 live-born infants had meconium-stained fluid. Thirty (2.1%) of these 1420 developed meconium aspiration syndrome and 12 (40%) died; eight received a postmortem examination. Four had unequivocal evidence of meconium aspiration, two had large numbers of intra-alveolar squamous cells, and two had no evidence of aspiration. We conclude that aggressive airway management during and immediately after delivery does not always prevent fatal meconium aspiration syndrome. (AM J OBSTET GYNECOL 1985;151:731-6.)

Key words: Meconium aspiration syndrome, postdate pregnancy, endotracheal suction

Meconium-stained amniotic fluid is present in 9% to 14% of all pregnancies at the time of delivery,[1, 2] although such staining occurs with increasing frequency as gestation advances. Indeed, the incidence can be as high as 44% in postdate pregnancies.[3] The passage of meconium, in most cases, is probably a physiologic event related to increasing maturity of the fetus; however, it is possible that it may at times occur as the result of fetal hypoxia and acidosis.[4] Increased perinatal morbidity and mortality rates have been associated with meconium-stained fluid.[5, 6] Of special concern is the meconium aspiration syndrome, which is initially manifested as respiratory distress, hypoxemia, and acidosis and which may progress rapidly to respiratory failure.[7] The reported neonatal mortality rate of meconium aspiration syndrome is $\geq 25\%$.[7, 8]

Traditionally, meconium aspiration has been thought to occur with the first respirations after birth. During the past decade, several reports have suggested that with appropriate airway management the incidence of meconium aspiration syndrome can be reduced greatly,[1, 2, 8] and that severe cases may even be preventable.[9] Specifically, the use of a combined obstetric and pediatric approach to tracheal toilet was associated with the absence of meconium aspiration syndrome–related deaths in a large service group over a 5-year period.[9] In 1976 we began using a protocol similar to this combined approach.[2] The protocol was to be used at the time of any delivery known or suspected to be complicated by meconium-stained amniotic fluid.[2] However, despite almost uniform adherence to this protocol, we have continued to see infants with severe meconium aspiration syndrome, some of whom have succumbed to this disorder. The purposes of this retrospective study were to evaluate the effectiveness of our protocol in preventing meconium aspiration syndrome and to determine the incidence of fatal and nonfatal meconium aspiration syndrome when this protocol was followed.

Material and methods

During the 5-year period from January 1, 1977, through December 31, 1981, all live-born infants delivered (vaginally or by cesarean section) through meconium-stained amniotic fluid at University Hospitals, Birmingham, Alabama, were to be treated as follows: as soon as the head was delivered but before delivery of the shoulders, diligent suction of the oropharynx and then the nasopharynx was performed with a DeLee trap. The delivery was then completed and the trachea intubated under direct vision by a member of the pediatric staff, at least one of whom was present at all cesarean deliveries and at every delivery when meconium had been detected or was suspected (no fluid found at amniotomy). Whenever meconium was ac-

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Table I. Data for live births during the 5 years of the study

Year	Live births (n)	Cases with meconium-stained amniotic fluid		Cases with clinical meconium aspiration syndrome		Deaths (n)	Mortality rate with meconium-stained amniotic fluid (%)	Mortality rate with clinical diagnosis of meconium aspiration syndrome (%)
		n	%	*n*	%			
1977	1359	144	10.6	5	3.5	2	1.4	40
1978	1816	265	14.6	6	2.3	2	0.8	33
1979	1998	288	14.4	5	1.7	2	0.7	40
1980	2211	399	18.0	6	1.5	4	1.0	67
1981	1915	324	16.9	8	2.5	2	0.6	25
Total	9299	1420	15.3	30	2.1	12	0.8	40

Table II. Data for 12 neonates who died with the clinical diagnosis of meconium aspiration syndrome

Case No.	Year	Birth weight (gm)	Gestational age at delivery (completed weeks)	Sex	1-min Apgar score	5-min Apgar score	Day of death
1	1977	3000	39	M	2	3	1
2	1977	2100	33	F	2	5	2
3	1978	2810	40	F	2	5	1
4	1978	3500	43	F	7	9	3
5	1979	3080	40	M	6	8	1
6	1979	3060	43	F	4	7	2
7	1980	2740	42	F	6	8	6
8	1980	2460	37	F	2	7	3
9	1980	3600	42	F	6	8	1
10	1980	3300	40	M	7	9	2
11	1981	3020	39	F	3	8	4
12	1981	3590	41	M	4	6	2

tually demonstrated with DeLee suction, the trachea was intubated and suctioned, regardless of whether meconium was seen above the vocal cords. This routine intubation is a modification of the approach described by Carson et al.,[2] who reserved intubation only for those infants with meconium visible on the vocal cords at the time of laryngoscopy. Repetitive intubations with endotracheal section were performed, if necessary, until the fluid returned in an endotracheal tube was not meconium stained. As another modification of the originally described combined method,[2] gastric aspiration was routinely performed after the endotracheal intubation(s). However, gastric aspiration was not done until the infant was acutely stabilized, and in addition, the heart rate was continuously monitored during the procedure. This protocol was followed uniformly except for infants delivered with breech presentation, those delivering precipitately in bed, and those whose mothers could not refrain from expelling the entire infant as soon as the head was born. These exceptions, while not receiving DeLee suction prior to the first breath, nonetheless did receive the endotracheal intubation(s) and endotracheal suction as described.

Deliveries that had been complicated by meconium-stained fluid were identified from the delivery-room log. No attempt was made to distinguish between thin and thick meconium. Deaths related to meconium aspiration syndrome were determined from the Neonatal Intensive Care Unit log and from reports of the Perinatal Mortality Committee. In addition, a list of newborn infants with the clinical diagnosis of meconium aspiration syndrome was obtained from the Medical Records Department, which uses the International Classification of Disease coding system. A retrospective mother-infant chart review was done for all cases in which neonatal meconium aspiration syndrome was identified. For purposes of this study, the clinical diagnosis of meconium aspiration syndrome was accepted only if there had been meconium-stained amniotic fluid, neonatal oxygen dependency (with or without ventilator dependency), and a chest radiograph that had been read as being consistent with meconium aspiration syndrome. Microscopic sections of lung from eight infants dying with a clinical diagnosis of meconium aspiration syndrome, along with several sections of lung from each of four neonates dying of other conditions and matched for gestational and postnatal ages, were reviewed by one of us (E. R. W.). The reviewer was unaware of the clinical circumstances surrounding each case. Special attention was given to the presence and location of any meconium or amniotic debris within the lungs of these infants.

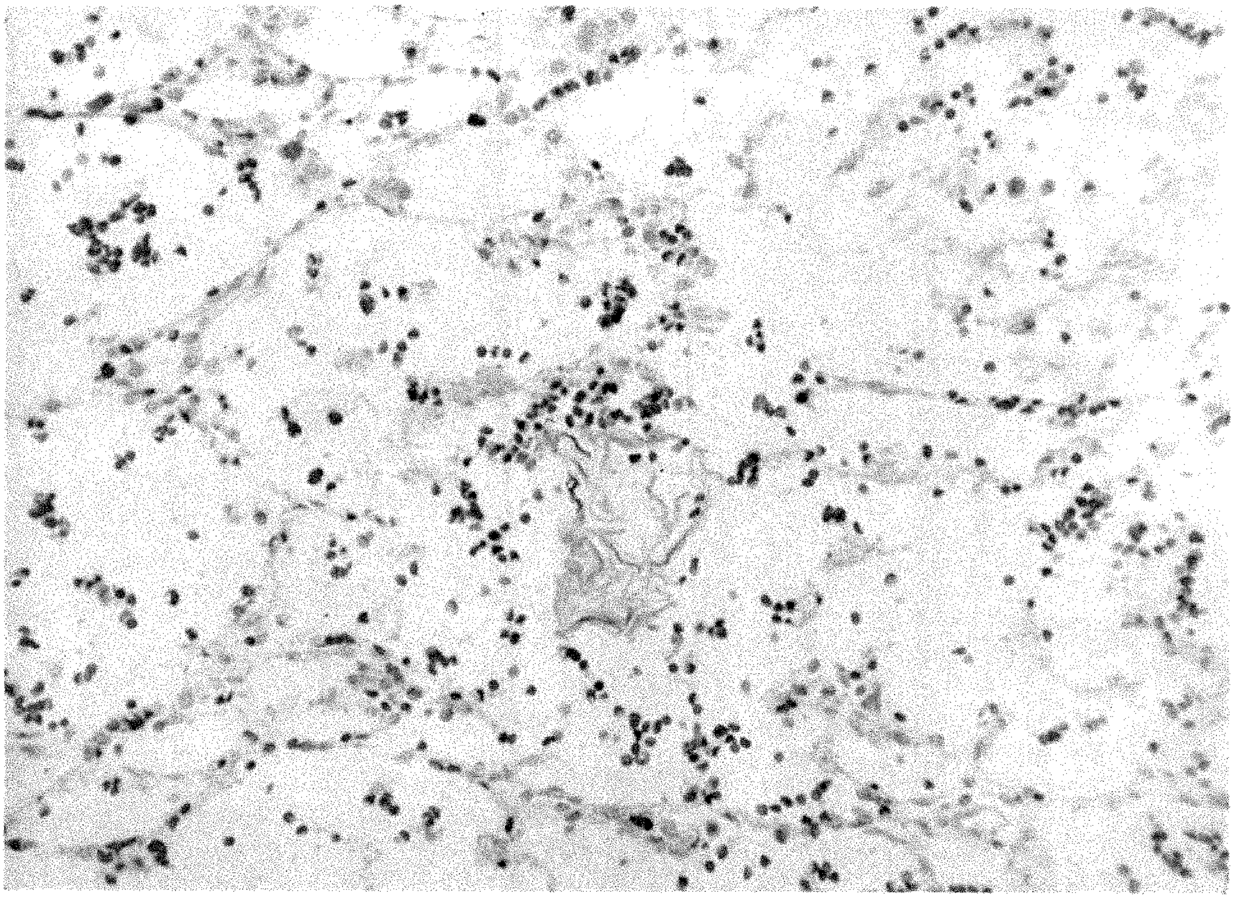

Fig. 1. Photomicrograph of lung of infant dying at 6 hours of age with meconium aspiration. Note golden-brown granular pigment of meconium, squamous epithelial cells, and erythrocytes within alveoli. (Hematoxylin and eosin stain. Original magnification × 100.)

Results

For each of the 5 years of the study, the total number of live births, the number with meconium-stained amniotic fluid at delivery, the occurrence of meconium aspiration syndrome, and the mortality rate associated with this syndrome are presented in Table I. Data regarding birth weight, gestational age, sex, Apgar scores, and postnatal day of death for the 12 neonates who died with the clinical diagnosis of meconium aspiration syndrome are presented in Table II.

No comparisons were made between infants who survived clinical meconium aspiration syndrome and those who did not. Nine of the 12 infants who died had persistent pulmonary hypertension of the newborn as one of the clinical diagnoses. It was diagnosed on the basis of profound and otherwise unexplained hypoxemia, normal intracardiac anatomy by sector scanning, and a marked difference in preductal and postductal arterial oxygenation (and/or evidence of a large left-to-right interatrial shunt by bubble-contrast echocardiography). Of the 12 neonates who died, three were delivered by cesarean section and three by indicated low-forceps operations. The indication for all six of these interventions was persistent fetal distress, diagnosed on the basis of fetal monitoring recordings showing recurrent late or severe variable decelerations, which was unresponsive to usual methods of intrauterine resuscitation. The other six infants showed transient episodes of these heart rate abnormalities but responded to intrauterine resuscitative measures and were delivered spontaneously. Ten infants had a 1-minute Apgar score of <7, and four of them had a 5-minute Apgar score of <7. One infant was not managed according to the protocol. This one infant (Case 4) was delivered precipitately in the labor room. DeLee suction was not used prior to delivery of the shoulders, and there was a delay of about 2 minutes before endotracheal suction was performed.

Eight of the 12 newborn infants who died underwent a postmortem examination. Four of them had unequivocal meconium aspiration, manifested by both meconium and fetal squamous cells in alveoli (Cases 3, 4, 7, and 9). One also had bacterial pneumonia (Case 7). Two other neonates had numerous alveolar squamous cells,

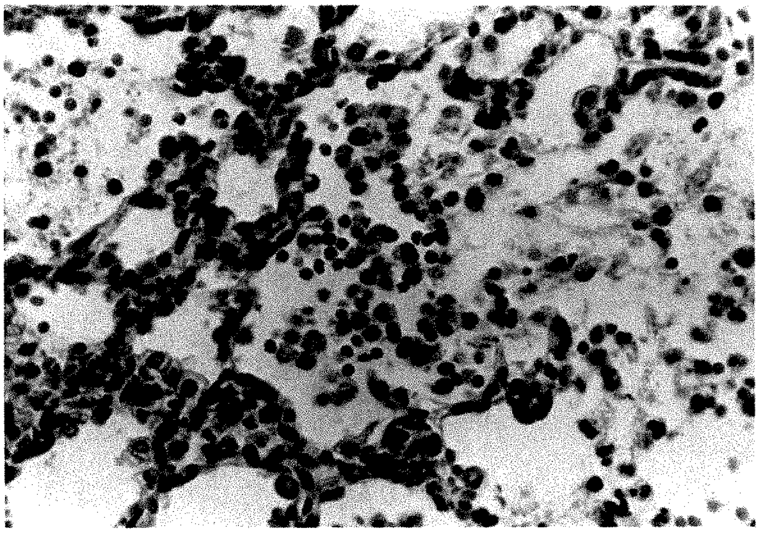

Fig. 2. Photomicrograph of lung of infant dying at 14 hours of age with meconium aspiration. In addition to meconium, alveoli contain erythrocytes and numerous polymorphonuclear leukocytes. (Hematoxylin and eosin stain. Original magnification ×400.)

but no meconium was noted (Cases 1 and 2). The only preterm infant dying with a clinical diagnosis of meconium aspiration syndrome (Case 2) had no evidence of hyaline membrane disease at autopsy. One infant had only a few squamous cells within alveoli (Case 12), and one had no evidence of having aspirated amniotic fluid (Case 5). Therefore, two infants had minimal or no evidence of amniotic fluid aspiration. On microscopic study of lung sections from the four neonates dying of other conditions, neither meconium nor squamous cells were observed.

Meconium in the lungs of infants with meconium aspiration syndrome who died at or before 2 days of postnatal age was characterized by yellow-brown pigment, appearing either as glossy globules or granular debris within airways and alveoli (Fig. 1). The intra-alveolar cellular response consisted of polymorphonuclear leukocytes and a few macrophages, some containing golden-brown pigment. The lungs of infants who died from meconium aspiration syndrome after 2 postnatal days had a prominent, exudative alveolar response consisting of abundant fibrin and hemorrhage (Fig. 2). Although polymorphonuclear leukocytes were still observed, phagocytic cells, many containing golden-yellow pigment, were more prominent. Atelectasis was observed in sections from the lungs of all four infants with unequivocal histologic evidence of meconium aspiration syndrome, as well as from the two infants with aspiration of only fetal squamous cells. In these two there was also an associated and prominent intra-alveolar inflammatory reaction, manifested by polymorphonuclear leukocytes, fibrin deposition, and hemorrhage.

Comment

Our retrospective review of 9299 live births revealed a 15.3% incidence of meconium-stained amniotic fluid at the time of delivery. The incidence of meconium aspiration syndrome was 2.1% of those infants born through such fluid, despite the described aggressive airway management. Although the mortality rate associated with meconium-stained fluid was only 0.8%, the mortality rate with the clinical diagnosis of meconium aspiration syndrome was 40%. Meconium aspiration was confirmed histologically in the lungs of half of the eight infants who died with a clinical diagnosis of meconium aspiration syndrome and on whom autopsies were done; aspiration was suggested for two others. Thus, vigorous airway management does not always prevent either meconium aspiration syndrome or death from meconium aspiration syndrome. This point has not been sufficiently emphasized.

In a prospective study, Gregory et al.[1] found an 8.8% incidence of meconium-stained fluid, with a 20% incidence of meconium aspiration syndrome in those infants born through meconium. Meconium was present in the trachea of 50% of these neonates, including about one sixth of those with no meconium visible on the vocal cords at laryngoscopy. Two thirds of the infants from whom meconium was recovered from the trachea had no clinical evidence of respiratory disease, although 50% had abnormal chest radiographs. All but seven infants breathed deeply before airways could be suctioned. Eight of the sick infants had meconium below the cords but none in the mouth or on the larynx. Routine endotracheal intubation and suctioning of meconium from the trachea was recommended to prevent severe meconium aspiration syndrome. Because of these findings of Gregory et al.[1] and because of the large numbers of both obstetric and pediatric house officers providing care at this institution, we have followed the protocol described, which (because of routine endotracheal suction) is even more aggressive than the combined method of Carson et al.[2]

Ting and Brady,[8] in a retrospective study of meconium-stained infants, indicated that immediate endotracheal intubation and suctioning of meconium from the trachea decreased morbidity and mortality rates from meconium aspiration syndrome. Ninety-seven of these infants received prompt endotracheal suction, 27 (28%) became symptomatic, but only one died; that one death was due to congenital heart disease. Of 28 infants who did not receive prompt endotracheal suction, 16 (57%) developed meconium aspiration syndrome and seven (25%) died.

Carson et al.[2] demonstrated a marked reduction in the frequency of meconium aspiration syndrome with their combined obstetric-pediatric method, which utilized endotracheal suction only if meconium was present on the vocal cords at laryngoscopy. In 273 meconium-stained infants managed with this combined approach, only one case of meconium aspiration syndrome occurred, an incidence of 0.4%. This was compared to a 1.9% incidence of meconium aspiration syndrome in a prior period when endotracheal suction was done without routine, prior to DeLee suctioning. Gage et al.[10] have also shown that catheter suction is more efficient in clearing the upper airway of meconium than is bulb suctioning. Curiously, the incidence of meconium aspiration syndrome in our study with the use of the protocol described was essentially the same as this 1.9% found by Carson et al.[2] without routine use of DeLee suctioning.

The issue of in utero aspiration of meconium-stained fluid as an etiology of meconium aspiration syndrome has been controversial. Carson and Bowes[9] reported that the incidence of fatal meconium aspiration syndrome during a 5-year period was zero when their combined obstetric-pediatric approach was used on a large clinical service. Gooding et al.[11] were unable to produce meconium aspiration syndrome from in utero gasping of meconium-stained amniotic fluid by fetal puppies. However, Block et al.[12] have demonstrated that baboon fetuses aspirate meconium-stained amniotic fluid when they are made acidotic, but hypoxemia alone does not produce meconium aspiration. From autopsies of human stillbirths, Brown and Gleicher[13] presented evidence that in utero aspiration of amniotic fluid, with or without meconium, may occur. Manning et al.[14] reported a case of fatal in utero aspiration of meconium. The infant was severely depressed at delivery, received endotracheal suction, and never developed spontaneous respiration. Death occurred shortly after delivery, and microscopic sections of the lungs showed meconium and fetal squamous cells in the alveoli. Intra-alveolar macrophages contained phagocytized meconium. These latter studies suggest that some cases of meconium aspiration syndrome may not be preventable, even by the most aggressive airway management at the time of delivery. It is probable that whenever meconium happens to be present in the amniotic fluid, fetal hypoxemia *plus* acidosis can result in gasping and consequent in utero aspiration of meconium.[4, 12] Since the acidotic fetus also has reduced pulmonary flow and consequent reduced production of pulmonary fluid, such in utero aspiration occurring remote from the time of delivery may not be negated by the usual outflow of this pulmonary fluid.[4]

A problem in the interpretation of this study and previous clinical studies concerning meconium aspiration syndrome is the relationship between meconium staining and persistent pulmonary hypertension of the newborn. Low Apgar scores and meconium staining are frequently associated with persistent pulmonary hypertension of the newborn.[15] This syndrome is present in the newborn infant whose pulmonary vascular pressure and resistance fail to decrease, leading to continued right-to-left extrapulmonary shunting. Considerable evidence suggests that aspiration of meconium can produce persistent pulmonary hypertension of the newborn.[16] However, many infants who die with this syndrome have structural abnormalities of the pulmonary arterioles that could only have arisen antenatally, presumably as a result of subacute or chronic hypoxia, hormonal imbalance, drug ingestion, or other factors.[17] Although the presence of meconium in the lungs of infants with structural vascular changes may exacerbate the severity of persistent pulmonary hypertension of the newborn, it may not be the primary etiologic factor. Thus even complete clearance of meconium from the tracheobronchial tree may not prevent respiratory disease. Unfortunately, we were unable to evalute the pulmonary vasculature of the infants who died with the clinical diagnosis of meconium aspiration syndrome. It is likely that at least two of our infants died from persistent pulmonary hypertension of the newborn. Thus some but certainly not all of the infants with suspected meconium aspiration syndrome in this and previous studies may actually have had persistent pulmonary hypertension of the newborn.

In summary, when compared with data reported by Gregory et al.,[1] the incidence of symptomatic meconium aspiration syndrome appears to be reduced by a combined obstetric and pediatric approach.[2, 9] Our results, however, do not support the contention that severe meconium aspiration syndrome can be prevented altogether. Rather, our data emphasize the continuing possibility of serious consequences from meconium aspiration syndrome.

Why did the protocol fail to prevent all cases of fatal meconium aspiration syndrome, contrary to the findings of Carson and colleagues?[2,9] There could be several explanations postulated. Technically, perhaps we were not sufficiently thorough in DeLee suctioning prior to delivery of the shoulders. This possibility seems unlikely, since only one of the 12 meconium aspiration syndrome deaths was associated with failure to follow the protocol exactly. Also, great emphasis was placed on correctly performing the DeLee suctioning, and a maternal-fetal medicine faculty member was physically present for all deliveries. Neonatal respirations often occur prior to laryngoscopy and endotracheal suction, possibly accounting for some cases of meconium aspiration syndrome. In view of recent reports,[12-14] a plausible argument can also be made for in utero aspiration, even prior to onset of labor. This would most likely occur during a severe fetal asphyxial episode at any time and could result in the aspiration of large volumes of meconium-stained fluid.[4, 12] Occurrence of such a mechanism, if true, would support a policy of more rapid intervention in cases of persistent intrapartum fetal distress in the presence of meconium. It should be emphasized that 50% (6/12) of our infants dying with clinical meconium aspiration syndrome had fetal heart rate abnormalities considered to reflect serious fetal compromise. In addition, the Apgar scores were generally low in these 12 infants (Table II), although the possible adverse effects of endotracheal suction, sometimes repetitive, on these scores must be considered.[18] Chronic or acute hypoxia could also account for changes in pulmonary vascular resistance resulting in persistent pulmonary hypertension of the newborn. Since many cases of meconium aspiration syndrome may be preventable by nasopharyngeal suction prior to delivery followed by endotracheal suction immediately after delivery, perhaps only the severe cases (possibly having their antecedents prior to or during labor) remain, thus accounting for the high mortality we observed.

Our results indicate that infants born through meconium-stained amniotic fluid require close observation in the immediate neonatal period and rapid intervention if respiratory distress occurs. Although adverse outcomes do occur with optimal airway management, vigorous tracheal toilet should be performed and appropriately documented in the medical record.

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Factors involved in the interpretation of fetal monitor tracings

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In order to study how physicians choose to use electronic fetal monitoring and interpret tracings, we administered a questionnaire to which 107 practicing obstetricians and 11 experts in electronic fetal monitoring responded. Sixty-one (57%) of the respondents monitored more than half of their deliveries (high users). In comparison to the less frequent users of electronic fetal monitoring (low users), they showed more positive attitudes toward electronic fetal monitoring and were nearly always more likely to perform cesarean sections on hypothetical patients described in the questionnaire. These differences appeared to be due to the high users' higher estimate of danger to the fetus. We also found that most physicians were generally more likely to perform a cesarean section on a high-risk mother than a low-risk mother with the same tracing. The majority of high and low users and nearly all of the experts, however, felt that antepartum risk factors are not of value in deciding what to do about an abnormal tracing. We conclude that there is wide variation in the way in which obstetricians use, interpret, and act on electronic fetal monitoring tracings. Some of these differences may be due to differing attitudes toward electronic fetal monitoring, differences in interpretation of electronic fetal monitoring tracings, and differences in the way obstetricians incorporate maternal risk factors into their decision-making. (AM J OBSTET GYNECOL 1985;151:737-44.)

Key words: Fetal heart rate monitoring, cesarean section

When electronic fetal monitoring was introduced into practice, many clinicians feared that labor and delivery would become too technical and that reliance on clinical judgment would be abandoned.[1, 2] Today it is generally agreed that knowledge of the fetal monitor signs of fetal distress is complete enough and useful enough to warrant their use in the management of many labors.[3-5] There is less agreement, however, on how monitor information should be used to select patients for intervention in labor. This controversy in management arose because abnormal fetal heart rate (FHR) patterns occur frequently and by themselves do not always indicate the need for termination of labor.[6-8] As a result, the rates of cesarean section and interventions associated with electronic fetal monitoring as well as the predictive value of monitor results vary greatly between studies.[5]

Few studies of electronic fetal monitoring have examined the variables which may affect the clinician's interpretation of (and actions on) abnormal tracings. Some experts feel that variation in both technical skill and knowledge of electronic fetal monitoring account for the variation in management and results.[8] Other factors which may have led to differences between studies are the timing of intervention,[3] different reliance on measurements of fetal acid-base status,[10] and variable attitudes of physicians.[11, 12] While these studies provide some insights, there is still very little known about the role of clinical judgment in deciding which patients with abnormal FHR patterns would benefit most from intervention.

In current obstetric practice, the earliest management decision the physician makes is whether to monitor the laboring patient. For physicians who do not monitor routinely, the patient's risk status is a strong determinant of whether the monitor is to be used. Neither the physician's use of routine fetal monitoring nor the risk status of the patient has been studied as a factor in the management of abnormal electronic fetal monitoring tracings. This study examines the impact of physician attitudes, antepartum patient risk factors, and the routine use of electronic fetal monitoring on the decision to intervene to terminate labor.

Methods

Study data came from a questionnaire sent to 418 Board-certified obstetricians. The names and addresses of most physicians were obtained from the 1982 edition of the *Directory of Medical Specialists*. Eight addresses were selected from local telephone books in various cities. Half of the questionnaires were mailed in March, 1982, and the other half in December, 1982.

Design and evaluation of the questionnaire. We designed the questionnaire with the help of faculty members of the Department of Gynecology and Obstetrics at Stanford University School of Medicine. The ques-

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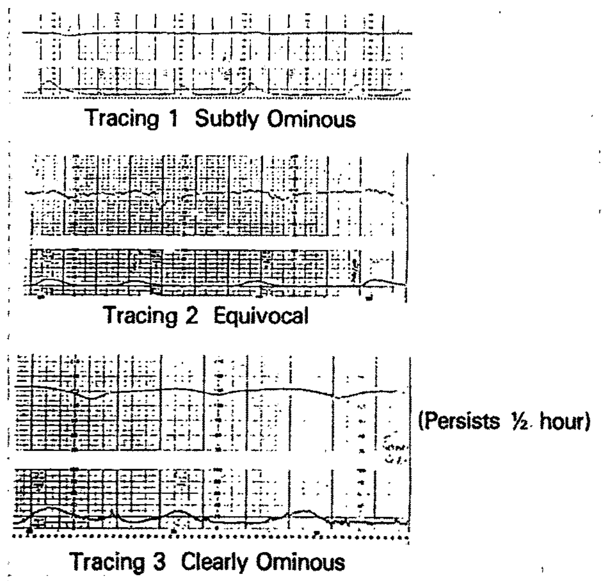

Fig. 1. Study tracings. Each of these fetal monitor tracings was used in conjunction with either a high-risk or low-risk maternal history in the study questionnaire.

tionnaire was pretested by the gynecology and obstetrics house staff of two large teaching hospitals in the area and by several practicing obstetricians in Palo Alto. We used their questionnaire responses and comments from personal interviews to eliminate unclear and unreliable questions.

After distribution of the final questionnaire, the comments of respondents on the questions were examined. Since many respondents were critical of the wording of two questions, we eliminated these two questions from the final analysis.

Content of questionnaire. The questionnaire had four sections. The first obtained data on the physician's training, practice setting, and use of electronic fetal monitoring. The second section assessed attitudes about the benefits of electronic fetal monitoring by using five-point agreement-disagreement questions. In the third section, the knowledge section, the physician was asked to indicate the prognostic significance of selected FHR patterns, to assess the usefulness of electronic fetal monitoring in various clinical situations, and to indicate his/her degree of agreement with some strategies for managing abnormal electronic fetal monitoring tracings.

The fourth section consisted of case histories. For each patient, physicians were asked to estimate the chance of death or damage to the fetus and to decide whether a cesarean section for fetal distress was indicated. Each case history presented one of three tracings and either a high-risk or low-risk patient history (Fig. 1). Each physician was given three of the six possible case combinations, one with each tracing, so that the effects of the patient factors, physician factors, and different tracings could be assessed independently.

The three tracings were selected to represent different degrees of abnormality. The first tracing shown was considered to be ominous but subtly so. The second tracing was considered equivocal and the third was clearly ominous.

Follow-up and nonrespondents. Information from the *Directory of Medical Specialists* was used to compare nonrespondents to respondents. In addition, an attempt was made to reach all addressees by telephone to detect reasons for nonresponse and to encourage those who had not yet received questionnaires. The comments of physicians who had seen the questionnaire were recorded. Nonrespondents were asked about their views and attitudes regarding electronic fetal monitoring and their current practice setting. Addressees who were retired or whose addresses were obsolete were identified.

Physician groups. Respondents were classified as either high users or low users of electronic fetal monitoring. Physicians who monitored more than half of their laboring patients internally composed the high-user group. Low users were defined as those who monitored less than half of their patients internally.

A separate sample of 11 fetal monitoring experts completed the questionnaire. All 11 of these physicians were chosen because they had published articles on electronic fetal monitoring and were felt by one of the investigators (K. U.) to be nationally known for their contributions to the field.

Data analysis. Physicians' responses were entered into a Statistical Package for the Social Sciences (SPSS) file and stored on disk. Statistics were calculated with the t test except for continuous and Likert-scale variables, for which nonparametric statistics (Kruskal-Wallis or Wilcoxon rank sum test) were calculated.

Results

A total of 115 questionnaires was returned. Eight of these were from physicians who were not currently practicing obstetrics. The remaining 107 responses were analyzed.

Reasons for nonresponse. The post office returned 10.5% of the questionnaires as undeliverable. In most of these cases the physician either was decreased or had relocated.

Of the remaining addressees, 55% were contacted directly or through their secretaries. Of those contacted, 12.1% had not responded because they no longer practiced obstetrics. Other reasons for nonresponse were vacation, relocation, and "not enough time." These responses were rare. Three physicians thought the questionnaire was "biased" or unfair but intended to respond.

Table I. Baseline comparisons of demographic and practice data for high users, low users, and experts in electronic fetal monitoring

	High users	*Low users*	*Experts*
No.	61	46	11
Age (mean ± SD)	46.224 ± 8.152	49.326 ± 10.167	40.545 +4.3442
Source of EFM training			
None, informal, or CME	34 (56%)	33 (72%)	
Residency	19 (31%)	11 (24%)	
Fellowship	8 (13%)	2 (4%)	11 (100%)
No. of deliveries per month (mean* ± SD)	32.123 ± 63.798	15.391 ± 13.924	76.545 ± 135.887
Teaching program in hospital (%)	44	39	100
Use of fetal scalp pH (%)	60.7	47.8	100
Cesarean section rate for fetal distress†	1.7	1.4	1.1
Cesarean section rate increased by EFM	27 (44%)	18 (46%)	0
Fetal scalp blood sampling available	45 (74%)	33 (71%)	11 (100%)
Hospital has policy of routine EFM†	31 (51%)	8 (17%)	8 (73%)
No. of labor and delivery beds (mean ± SD)	17 ± 22.9	18 ± 24.2	25.7 ± 29.7
High-risk patients in practice (%)	22	20	80
No. of residents in program (mean ± SD)	7.7 ± 9.6	8.9 ± 10.8	20.5 ± 15

EFM = Electronic fetal monitoring; CME = continuing medical education.

*Several physicians indicated >100 deliveries per month, presumably because they supervise others (house staff) who perform deliveries.

†Indicates probability range as follows: 1 = 0% to 5%, 2 = 5% to 20%, 3 = 10% to 20%, 4 = 40%.

†Difference between high and low users is significant ($p < 0.01$).

On the average, respondents were younger than nonrespondents because of the large number of retired or deceased nonrespondents. Nonrespondents were more likely to limit their practice to gynecology. Many physicians who had retired from obstetrics within the past decade voiced strongly negative views toward fetal monitoring. Other than these, there were no detectable sources of response bias.

Characteristics of respondents. There were 61 high users (57%) and 46 (43%) low users. High users and low users were similar in age, education, patient population, and practice size and setting (Table I). High users were significantly more likely to practice in hospitals that have a policy of routine fetal monitoring. They were somewhat more likely than low users to use fetal scalp pH sampling in their practice.

The expert group was younger than the sample obstetricians. They attended more deliveries on average although this difference is probably because they were more likely to supervise house staff. All experts worked in teaching hospitals with a policy of routine fetal monitoring. Scalp pH sampling was used by all experts compared to 60.7% of high users and 47.8% of low users. None of the experts felt that electronic fetal monitoring had increased their own cesarean section rate, while about 45% of both high and low users thought that it had.

Attitudes toward electronic fetal monitoring. While the respondents were generally enthusiastic toward electronic fetal monitoring, there were significant differences in attitudes between high and low users (Table II). High users were significantly more likely to support routine monitoring. High users had higher agreement scores on every statement favorable to fetal monitoring, while low users were significantly more likely to believe that electronic fetal monitoring interferes with the normal process of labor and delivery. Experts had more enthusiasm for electronic fetal monitoring than high or low users. Only one of 11 experts thought that electronic fetal monitoring interferes with the normal labor and delivery process.

Table II. Opinions of obstetricians concerning fetal monitoring

	High users (n = 61)	*Low users (n = 46)*	*Experts (n = 11)*
1. EFM is better than auscultation for low-risk patients (% agreeing)	90	78	73
2. EFM has led to lower perinatal mortality (% agreeing)	79	74	82
3. EFM has led to higher cesarean section rate (% agreeing)	75	85	73
4. EFM interferes with labor and delivery (% agreeing)	15	37	9
5. EFM should be routine in all deliveries (% agreeing*)	82	36	91

EFM = Electronic fetal monitoring.

*High users and experts significantly higher ($p < 0.05$) than low users.

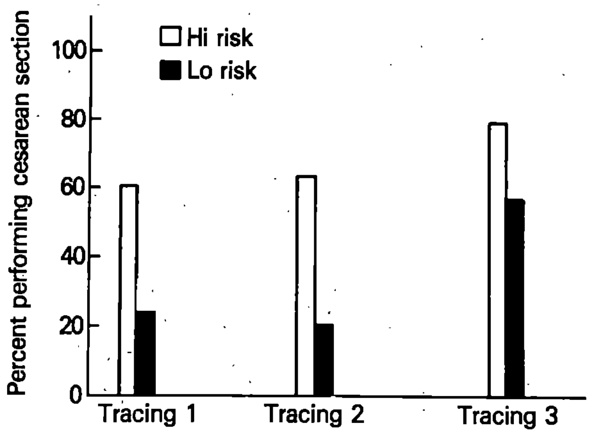

Fig. 2. Response to clinical scenarios. The ordinate shows the six different clinical scenarios (three different electronic fetal monitoring tracings and two different risk categories). The abscissa shows the proportion of physicians indicating they would perform a cesarean section for each scenario. The difference between the high- and low-risk patient is statistically significant ($p < 0.01$, χ^2 test) for all three tracings.

Table III. Knowledge comparisons

	High users	*Low users*	*Experts*
1. Antepartum risk factors are of little value in evaluating ominous fetal heart patterns (% agreeing)	67	54	82
2. The correct diagnosis of fetal distress is confirmed by a low 1-minute and high 5-minute Apgar score (% agreeing)	33	39	20
3. How often do you allow labor to continue despite significant periodic deceleration or prolonged bradycardia? (% <40% of the time)	50	54	72.7

Attitudes that may be related to management strategies for abnormal tracings are shown in Table III. Most high and low users and nearly all experts felt that antepartum risk factors are not of value in deciding what to do about an abnormal tracing. The relationship of this belief to the actual behavior of physicians is discussed below.

Table III also demonstrates wide differences between experts and other obstetricians on issues that may affect selection of patients for interventions in labor. Experts are less likely to expect a baby who has been delivered by cesarean section for fetal distress to have a low 1-minute and a high 5-minute Apgar score. In addition, experts' estimates of the accuracy of their own diagnosis of fetal distress were lower than those of other physicians.

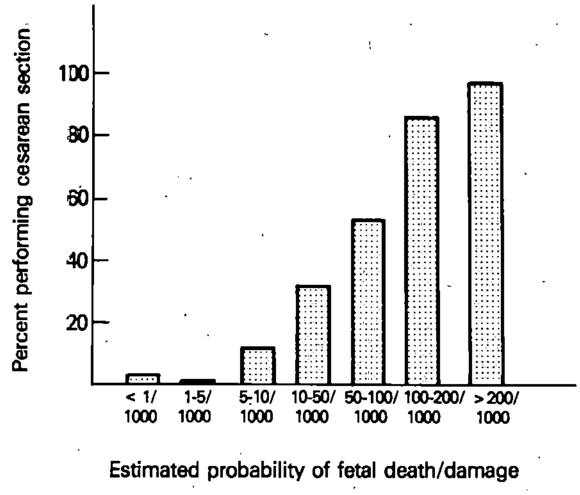

Fig. 3. Correlation of physician estimate of the probability of fetal death or damage and the decision to perform a cesarean section. The ordinate shows the estimates of physicians of the probability of fetal death or damage from the six clinical scenarios. The abscissa indicates the proportion of physicians in each probability category who would perform a cesarean section. Thus almost none of the physicians who indicated that the chances of danger were <5 per 1000 would perform a cesarean section, while nearly all of those who estimated the risk to be >100 per 1000 would do so.

Responses to clinical scenarios. Within each of the six scenarios, there was great disagreement over the need to intervene. Fig. 2 shows the proportion of all of the study physicians who would perform cesarean section for each scenario. Among the sample physicians (nonexperts), disagreement was greatest for three scenarios: the low-risk–ominous tracing scenario, which had a 54% cesarean section rate; the high-risk–subtly equivocal tracing scenario (61%); and the high-risk–ominous tracing scenario (64%).

The most striking finding depicted in Fig. 2 is the powerful effect of the clinical description on the decision to perform cesarean section. For a given tracing, a high-risk patient is significantly more likely to undergo cesarean section than a low-risk patient.

Risk factors had the strongest effect in the scenarios with an equivocal tracing and a weaker effect when the tracing was seen as clearly ominous. Hence for tracing 2 treatment was conservative when the patient was low risk (20% cesarean section rate) but aggressive when the patient was high risk (61% cesarean section rate, $p < 0.01$). The effect was similar for tracing 1 but smallest for tracing 3, which was seen as the most ominous.

Opinions on the danger to the fetus. When we compared them for all physicians and all scenarios, estimates of the danger of death or damage to the fetus were highly correlated with the proportion of physicians who chose to perform cesarean section (Fig. 3). Thus very few physicians chose to intervene when the probability of death or damage was estimated at <5%;

nearly everyone chose to intervene when the probability of death or damage was estimated to be >10%.

Differences between high and low users. High users had higher cesarean section rates than low users for five of the six scenarios. Fig. 4 shows the proportion of experts, high users, and low users who would perform cesarean section for each scenario. The biggest difference between high and low users was seen with the first two scenarios, which involved the subtle, ominous tracing. High users were significantly more likely than low users to perform cesarean section in the high-risk patient with tracing 1 than were low users (78% versus 33%, $p < 0.001$). Thus a significant portion of the overall disagreement on this tracing was related to the difference between high and low users. High users were also more likely to perform cesarean section in both scenarios involving tracing 3, the other ominous tracing. High and low users responded similarly to tracing 2, which both groups viewed as equivocal.

High users, like experts, considered tracings 1 and 3 to be more ominous than tracing 2. Low users, however, considered tracing 2 to be worse than tracing 1; in addition, they were significantly more likely to perform cesarean section in the patient with tracing 3 than the patient with tracing 1. The low users' rates of cesarean section for tracings 2 and 3 were not significantly different.

Like high users, experts found tracings 1 and 3 to be more ominous than tracing 2. However, while both high and low users were strongly influenced by risk factors in their decisions on tracing 1 (subtle, ominous), experts were not. As mentioned above, experts interpreted only tracing 2 as equivocal, while high and low users behaved as if all three tracings were equivocal to some degree.

Comment

In the present study, we identified two factors in decision-making with abnormal electronic fetal monitoring tracings: the risk status of the patient and the physician's attitudes regarding electronic fetal monitoring. These findings provide additional direction to investigations into the sources of physician disagreement over the management of patients with abnormal cardiotocograms.

Role of patient risk factors. In our study, obstetricians appear to rely heavily on patient risk factors when deciding what to do about a selected FHR pattern, despite statements in the literature and on the attitude part of our questionnaire to the contrary. Thus the study obstetricians were almost twice as likely to perform cesarean section in a high-risk patient as in a low-risk patient with the same tracing.

Many experts feel that FHR patterns can by themselves show whether the fetus is tolerating hypoxic stress and thereby guide management.[7] Others believe that deterioration of fetal acid-base balance is a more specific indicator of the need to terminate labor.[13, 14] Both of these management strategies stress the pathophysiologic derangements that are associated with monitor signs. After known causes of "false positive" results are ruled out, the persistence of the derangements is considered sufficient reason to intervene in labor.

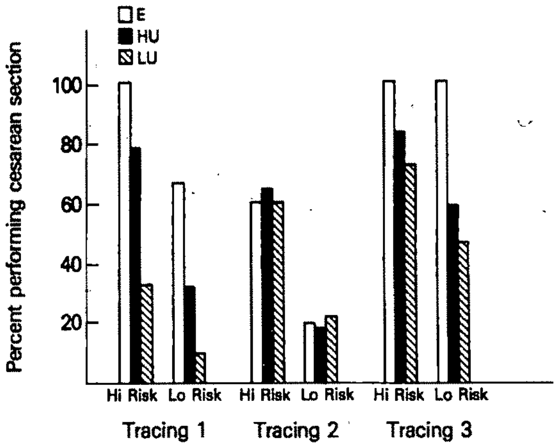

Fig. 4. Responses of high users *(HU)*, low users *(LU)*, and experts *(E)* to clinical scenarios. The ordinate shows the six different clinical scenarios and the abscissa shows the proportions of physicians indicating they would perform a cesarean section for each scenario. The *open bars* are the responses of "high-user" physicians; the *closed bars*, the responses of "low-user" physicians; and the *hatched bars*, the responses of experts.

The behavior of the physicians in our study is consistent with another approach, in which a tracing's ability to predict danger to the fetus also depends on the prior chance of danger that can be expected from the mother's history. According to this "predictive value" model, the attachment of a single, immutable prognosis to a particular pattern may be misleading. Instead, an abnormal pattern, such as tracing 2 in our study, may be seen as reassuring in one patient and as indicating the need for cesarean section in another.

Existing clinical data provide a few clues to which of these two approaches is better. Shenker[15] found that the predictive value of late decelerations for Apgar scores was twice as high for high-risk as for low-risk patients. Miller et al.[16] found that late decelerations were more predictive of low Apgar scores in patients with meconium-stained liquor. Kuhn[17] verified this finding using neonatal depression as an outcome measure. Westgren et al.[18] found that the predictive value of various FHR abnormalities depends on the gestational age of the fetus. Finally, Freeman et al.[19] demonstrated that the likelihood of late decelerations and fetal distress during labor, as well as the likelihood of low Apgar scores, depended on antepartum risk status as determined by risk factors and antepartum FHR monitoring results.

Unfortunately, most studies of the predictive value of electronic fetal monitoring signs have not been designed to examine high- and low-risk patients separately. Moreover, the proportion of patients with abnormal FHR patterns and with poor outcomes has varied widely in these studies, suggesting that population differences (and thus the prevalence of various risk factors) may play a role in the variability between study results. Many of these studies deliberately excluded consideration of the risk status of the patient from management decisions, so that it is impossible to judge whether such consideration is clinically valuable.

Ideally, one would like to know whether fetuses with equally abnormal FHR patterns have different prognoses, depending on what risk factors are present. Krebs et al.[20] found similar Apgar scores for high-risk and low-risk newborn infants who had similar FHR scores prior to delivery. Their analysis excluded operative deliveries, however, and did not look at specific risk factors individually. Moreover, the same study found that babies delivered vaginally did better than those delivered by cesarean section, despite similar FHR scores, especially when FHR abnormalities were mild or moderate. These results suggest that strategies for managing labor with electronic fetal monitoring are probably not optimal but do not answer the question of how risk factors should affect the decision to terminate labor.

Despite the need for accurate predictors of the need for intervention, clinicians are faced with the fact that nonreassuring and ominous tracings occur in up to 40% of monitored labors.[7, 21] Since only a small proportion (3% to 25%) of patients with such FHR patterns undergo cesarean section,[22] clinicians must rely on other means of deciding about intervention. Despite the paucity of data regarding specific risk factors, obstetricians in our study respond to those uncertainties by applying the "predictive value" model to the interpretation of electronic fetal monitoring information. The predictive value of a finding (defined as the proportion of abnormal results that represent true abnormalities) varies directly with the likelihood of an abnormality (as defined by the number and kind of risk factors) before electronic fetal monitoring is applied. Thus it is likely that obstetricians perform cesarean sections in high-risk patients with less specific (nonreassuring but not ominous) patterns but will not intervene in low-risk labors until more drastic, clearly ominous patterns are seen.

Such a management strategy has very different implications from one that depends solely on electronic fetal monitoring information. For example, as Shifrin[8] noted, a strategy which waits for the appearance of only the most ominous patterns in low-risk patients may permit fetal decompensation to occur before intervention occurs. Moreover, the strategy of acting upon less ominous patterns in patients with more risk factors may unnecessarily increase the cesarean section rate in this group. To resolve this question, more attention to the impact of fetal monitoring in different clinical situations is needed. More important, perhaps, is the need to recognize and evaluate different clinical responses to abnormal FHR patterns in patients with specific antepartum risk factors.

Role of physician attitudes. The second finding of this study is that self-identified high users of electronic fetal monitoring differ significantly from low users in their attitudes toward electronic fetal monitoring and in their responses to clinical scenarios. This finding supports the conclusion that variation in physician attitudes is associated with variation in the interpretation of fetal monitor tracings.

In this study, large disagreement over the need for cesarean section was observed in three of the six scenarios. These three scenarios can be characterized as an ominous tracing in a low-risk patient, a subtly ominous tracing (presumably perceived as an equivocal tracing) in a high-risk patient, and an equivocal tracing in a high-risk patient.

As mentioned in the introduction to this paper, suggested sources of disagreement in the management of labor include monitoring technique, use of scalp pH testing, and different levels of skill in interpreting tracings.[8, 9] Other possible factors are different experience regarding the prognosis of certain patterns, different philosophies and attitudes about fetal monitoring, and differences in attitudes about the risks and benefits of early intervention in labor.

Of these factors, only interobserver variability in reading electronic fetal monitoring patterns has been formally studied. Cohen et al.[11] found that electronic fetal monitoring experts agreed on the need for intervention for various FHR patterns 59% to 69% of the time. Beaulieu et al.[23] found even greater disagreement among five experienced obstetricians who reviewed 150 cardiotocograms. In their study, the proportion of tracings assessed as normal ranged from 39% to 75%, and the proportion assessed as abnormal from 3% to 43%. Neither of these studies formally tested knowledge, technical skill, or other factors which might account for disagreement. However, Cohen et al.[11] felt that their results supported the role of variation in the degree of interventionism as an explanatory factor. Using a different approach, Hall and Alexander[12] studied three practices with different indications for monitoring. The three groups had very different cesarean section rates despite similar patient populations and perinatal outcomes. The authors concluded that physician attitudes and clinical judgment accounted for the observed differences.

Our study verifies a role for physician attitudes in disagreement over which patients require cesarean section. Low users of electronic fetal monitoring tended to have lower cesarean section rates for both low-risk and high-risk patients with ominous tracings. We found no significant differences in training, practice, or knowledge between high and low users. However, the obstetrician's choice of whether to monitor routinely (high users) or selectively (low users) was associated with significant differences in attitudes regarding the benefits and costs of electronic fetal monitoring.

On the other hand, our data suggested that these attitudinal differences did not extend to disagreements about the value of the cesarean section. In Fig. 3, we show that the study physicians had a high degree of agreement between the probability of fetal death or damage and the need for cesarean section. The degree of agreement was the same for both high and low users. Thus variation in the physicians' decisions to intervene was due to variations in their assessment of the seriousness of the electronic fetal monitoring tracings rather than to variations in their assessment of the value of the intervention.

The low cesarean section rate among low users for the first tracing stands in distinct contrast to the rates at which high users and experts chose cesarean section, and this raises questions about the appropriateness of the low users' choices in this scenario. The roles played by knowledge and skill in evaluating tracings cannot be judged by our results. However, the cesarean section rates observed raise the possibility that existing strategies do not enable many obstetricians to optimally select patients for intervention. Because of the necessity to monitor from 1000 to 10,000 labors to prevent one hypoxic death,[3] it is important that the obstetrician select for intervention all patients who might benefit from termination of labor. Given the wide variation in electronic fetal monitoring interpretation seen in this study, the clinician's ability to identify the most ominous tracings should be the subject of further studies.

A weakness of this study is the small number of tracings examined. Cardiotocograms are complex and varied records, and a small selection cannot be taken as representative of all abnormal tracings. In selecting the tracings, it was intended that a range of abnormal tracings be compared. The proportion of abnormal tracings which fall into one of these three categories may be small. The results, therefore, cannot be considered to reflect the physicians' responses to other combinations of FHR patterns or their ease of interpretation. A second weakness is the reliance on self-reported, simulated responses to clinical information. This study shares the problems of all case history simulations. The conclusions of this study must, therefore, be tested in practice before they are accepted.

It may be added that a survey of obstetric opinion can only be a preliminary step in the study of observer disagreement and management strategies. The findings of this study should be seen as evidence of the uncertainty that remains in the clinical application of fetal monitor information. An appropriate response to such uncertainty would be to identify the sources of disagreement and compare, in the clinical setting, the results of different strategies for reducing this uncertainty.

Certainly, our study should add impetus to the need to study the role of antepartum risk factors and physician attitudes in obstetric decision-making. Such studies would lead to a clearer understanding of the important interactions between clinical and human factors as they affect one of the most important decisions an obstetrician can make.

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Intrapartum fetal heart rate monitoring of the extremely premature fetus

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When cesarean section is not warranted because of extreme prematurity, continuous electronic fetal heart rate monitoring during the intrapartum period and in utero resuscitation of the distressed fetus are indicated, especially in cases of borderline fetal viability. Two cases are presented to illustrate the benefit of such measures when fetal distress occurs and the fetus is judged to be too small for operative intervention. (AM J OBSTET GYNECOL 1985;151:744-5.)

Key words: Fetal heart rate monitoring, premature fetus

The decision for operative delivery in cases of extreme prematurity and fetal distress is usually based upon the estimated gestational age, fetal weight, and the corresponding neonatal survival rate of the institution where the neonate is born. This report pertains to two cases of borderline extreme prematurity where the judgment was made not to subject the mother to operative intervention for fetal reasons, because the chances for neonatal survival were judged to be less than reasonable.

Case reports

Case 1. A 29-year-old white woman, gravida 5, para 1, abortions 3, with a last menstrual period on October 4, 1983, was referred to the University of Connecticut Health Center at 23½ weeks' gestation because of premature rupture of the membranes.

Ultrasound examination revealed a singleton fetus in a vertex presentation. The biparietal diameter, head circumference, abdominal circumference, and femur length were all compatible with 23 to 24 weeks' gestation. The estimated fetal weight[1] determined by ultrasonography was 600 gm.

On the second hospital day, the patient developed chorioamnionitis and the decision for delivery was made. The decision was also made not to subject the mother to cesarean section in the event of fetal distress, because the fetus was judged to have a less than reasonable chance for survival because of the extremely low gestational age and estimated weight. However, it was felt that the fetus should be given the benefit of continuous electronic fetal heart rate monitoring and supportive medical therapy (changes in maternal position, oxygen administration) to minimize hypoxia if fetal distress developed. Early in the course of labor the fetus exhibited deep variable decelerations which required frequent maternal positioning, oxygen administration, and careful adjustments of the administered oxytocin. This treatment resulted in a normal fetal heart rate tracing throughout the active phase of labor. After 10 hours of labor a male infant weighing 630 gm was delivered over a midline episiotomy, with Apgar scores of 3 and 7 at 1 and 5 minutes, respectively. The cord blood gas values were reported to be as follows: pH = 7.34, P_{O_2} = 22.2 mm Hg, P_{CO_2} = 43.3 mm

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Hg, and base excess = −4 mol/L. The infant subsequently did very well with no major complications and is alive and well at the time of this writing. The mother was discharged on the third postpartum day in good condition.

Case 2. A 23-year-old black woman, gravida 1, para 0, with a last menstrual period on September 10, 1982, was referred to the University of Connecticut Health Center at 23 weeks' gestation because of premature labor, rupture of the membranes, and vaginal bleeding. On admission, the cervix was dilated 4 cm and uterine contractions were present every 2 to 3 minutes. Ultrasound examination was compatible with a singleton fetus in a vertex presentation. The biparietal diameter, head circumference, abdominal circumference, and femur length were all compatible with approximately 23 weeks' gestation and the estimated fetal weight was 610 gm. Internal fetal heart rate monitoring revealed the presence of deep variable decelerations with uterine contractions occurring every 2 to 3 minutes. The fetus was judged to be very small for an operative delivery, but intensive in utero resuscitation was started with oxygen administration and frequent maternal positional changes including knee-chest position. The fetal heart rate tracing improved, and 5 hours later a female infant, weighing 640 gm, was born over a midline episiotomy with Apgar scores of 4 and 7 at 1 and 5 minutes, respectively. The cord blood gas values were as follows: pH = 7.28, P_{O_2} = 16 mm Hg, P_{CO_2} = 30 mm Hg, and base excess = −6 mol/L. The infant had no major complications in the neonatal period and was discharged alive and well at 4 months of age. The mother was discharged on the second postpartum day in very good condition.

Comment

For the past decade, there has been a significant improvement in neonatal mortality and morbidity following preterm delivery, especially in the very low–birth weight infant (<1500 gm).[2] The main factors responsible for this improved outcome are the aggressive neonatal approach for resuscitation, ventilation, and intensive care for the preterm infant as well as the availability of fetal assessment by electronic fetal heart rate monitoring during the antepartum and intrapartum periods. However, there always seems to be a limit dependent upon gestational age, estimated fetal weight, and intensive care nursery facilities below which aggressive management with cesarean section is not warranted. In such borderline cases, electronic fetal heart rate monitoring should not be abandoned as has been the usual practice by many physicians. Our cases illustrate the significance of continuous electronic fetal heart rate monitoring and medical treatment with in utero resuscitation if fetal distress occurs during labor. In spite of evidence of fetal distress early in the course of labor, frequent changes in maternal position, oxygen administration, and cautious administration of oxytocin resulted in normal fetal heart rate tracings throughout most of the active phase of labor and normal cord blood pH in both cases. The in utero fetal resuscitation by these means should not be denied to the extremely premature fetus just because the decision was made not to subject the mother to cesarean section. In view of the tendency to underestimate fetal weight in preterm gestations, electronic fetal heart rate monitoring and supportive measures to minimize fetal distress become imperative in the intrapartum management of such borderline cases. Nontreatment or failure to provide simple supportive measures to the distressed fetus in cases of borderline extreme prematurity could potentially expose the responsible physician to medical and legal ramifications.

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Subfractions of high-density lipoprotein cholesterol during estrogen replacement therapy: A comparison between progestogens and natural progesterone

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Subfractions of high-density lipoprotein cholesterol and its apolipoproteins were followed up in 58 postmenopausal women during three cycles of unopposed estrogen replacement therapy with 2 mg of estradiol valerate daily. During the last 10 days of the following three cycles the women received sequential addition of either 250 μg of levonorgestrel, 10 mg of medroxyprogesterone acetate, or 200 mg of natural micronized progesterone. Both progestogens significantly decreased total high-density lipoprotein cholesterol as well as subfraction 2 of high-density lipoprotein. Data suggest that doses and relative biologic activity of 19-norsteroids and 17-hydroxyprogesterone derivatives are more important for their metabolic effects than are qualitative differences. Natural progesterone had no apparent influence on high-density lipoprotein cholesterol or its subfractions and may develop into an attractive alternative to synthetic progestogens. (AM J OBSTET GYNECOL 1985;151:746-50.)

Key words: Menopause, high-density lipoprotein cholesterol, estrogen, progesterone

Epidemiologic data suggest that estrogens may reduce the risk of atherosclerosis and ischemic heart disease after menopause.[1] This effect has been related to alterations of the serum lipoprotein profile and especially to the increased concentrations of high-density lipoprotein cholesterol during therapy.[2, 3] During estrogen replacement therapy the sequential addition of a progestogen is currently recommended. Several studies have demonstrated that progestogens are protective against excessive endometrial proliferation.[4, 5] In clinical practice two main groups of synthetic progestogens are used to mimic the effects of natural progesterone. While 19-norsteroids such as norgestrel and norethindrone are known to reverse the effects of estrogens on lipoprotein metabolism and to decrease high-density lipoprotein cholesterol, medroxyprogesterone acetate, which is a 17-hydroxyprogesterone derivative, is generally considered to have no such effect.[6-8] Recently an orally active preparation of micronized progesterone has become available, which theoretically might offer an alternative treatment with certain metabolic advantages.[9, 10] In the present study the effects of different progestogens and micronized progesterone on the cholesterol fraction of high-density lipoprotein, its subgroups, and apolipoproteins were compared during postmenopausal replacement therapy.

Material and methods

Subjects. A total of 58 women aged 46 to 69 years (mean age 53 years) attending the clinic because of typical symptoms of estrogen deficiency were recruited for the study. All of the women had had their last menstrual period at least 6 months earlier and all had increased serum follicle-stimulating hormone (FSH) values. They were apparently healthy and without any medication for the previous 3 months.

Hormonal treatment. All of the women were primarily treated with tablets of 2 mg of estradiol valerate daily. Treatment was prescribed cyclically for 3 weeks with an interval of 1 week. After the third cycle they were randomly allocated to three groups for sequential addition of different progestogens. Progestogens were given during the last 10 days of each treatment cycle. Twenty women received 250 μg of levonorgestrel daily, 20 women received 5 mg of medroxyprogesterone acetate twice daily, and 18 women were given 100 mg of micronized progesterone (Laboratoires Besins Iscovesco, Paris, France) twice daily.

Venous blood samples were drawn before therapy was started and subsequently at clinical examinations during the last days of the third, fourth, and sixth cycles. Serum was collected and stored at −20° C until analyzed.

Serum lipid and lipoprotein analyses. Determinations of total serum cholesterol and the cholesterol content of high-density lipoprotein and subfraction 3 of

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Table I. Serum levels (mean ± SEM) of FSH, total plasma cholesterol, high-density lipoprotein cholesterol, its subfractions, and main apolipoproteins in 58 postmenopausal women before and after 3 months of cyclic replacement treatment with 2 mg of estradiol valerate daily

Serum concentrations measured	*Before treatment*	*Third cycle*
FSH (U/L)	72.32 ± 5.01	51.47 ± 4.08*
Total cholesterol (mmol/L)	6.67 ± 0.12	6.30 ± 0.11*
High-density lipoprotein cholesterol (mmol/L)	1.20 ± 0.04	1.31 ± 0.04*
Subfraction 2 of high-density lipoprotein cholesterol (mmol/L)	0.63 ± 0.04	0.73 ± 0.04*
Subfraction 3 of high-density lipoprotein cholesterol (mmol/L)	0.56 ± 0.01	0.58 ± 0.02
Apolipoprotein AI (%)	92.36 ± 2.14	100.65 ± 2.18*
Apolipoprotein AII (%)	97.02 ± 2.55	103.71 ± 3.23†

$^*p<0.001$.
$^\dagger p<0.01$.

high-density lipoprotein were performed enzymatically with a cholesterol oxidase-peroxidase technique with a commercially available kit (CHOD-PAP kit 187318) from Boehringer Mannheim (Houston, Texas).

High-density lipoprotein cholesterol was determined after removal of very–low-density lipoprotein and low-density lipoprotein by precipitation with heparin–manganese chloride. Subclass 3 of high-density lipoprotein was obtained by further precipitation of subfraction 2 high-density lipoprotein from high-density lipoprotein with dextran sulfate (mean molecular weight 15,000) as described by Gidez et al.[11] The level of subfraction 2 high-density lipoprotein cholesterol in serum was obtained by subtracting subfraction 3 high-density lipoprotein from high-density lipoprotein cholesterol.

Analyses of A apolipoproteins. Serum apoprotein AI levels were determined by electroimmunoassay. A monospecific rabbit antiserum to human apoprotein AI was used. The immunoelectrophoretic runs were performed in Tris-barbital buffer, pH 8.6, overnight at a potential gradient of 2 V/cm.[12] All samples from individual women were run on the same plate. Pooled plasma from healthy blood donors was used as standard. The relative levels of apoprotein AI were expressed as a percentage of the plasma standard.

Serum apoprotein AII levels were determined in the same way with a monospecific rabbit antiserum to human apoprotein AII with the exception that 3% (w/v) polyethylene glycol (molecular weight 6000) was included in the agarose gel in order to obtain more distinct immunoprecipitates.

Statistical methods. Statistical significance was assessed by Student's *t* test for paired samples and Wilcoxon's signed rank test. Values of $p < 0.05$ were considered statistically significant.

Results

Clinical observations. All of the women reported subjective relief from vegetative symptoms such as hot flashes and sweats. No changes in body weight or blood pressure were recorded. All of the women who were treated with estradiol valerate and synthetic progestogens and who had a uterus had small to modest withdrawal bleeding during the week off treatment. Two of these 40 women had previously undergone hysterectomy for benign conditions. Of the 18 women receiving micronized progesterone, prior to the study one woman underwent removal of the uterus because of myoma. Ten women had regular and two women occasional withdrawal bleeding, whereas five noticed no bleeding at all during the course of the study.

Laboratory data. Unopposed estrogen replacement therapy of 58 women with 2 mg of estradiol valerate daily significantly increased the mean serum concentrations of high-density lipoprotein and subfraction 2 high-density lipoprotein cholesterol and the relative concentrations of the apolipoproteins AI and AII. Levels of FSH and total cholesterol declined while subfraction 3 of high-density lipoprotein cholesterol was unchanged (Table I). Among the various subfractions, the second subfraction of high-density lipoprotein cholesterol was relatively the most sensitive to the estrogenic effect.

When the women had been allocated to three groups for progestogen addition, there were no significant differences with respect to age, FSH values, or lipoproteins between the two subgroups treated with levonorgestrel and medroxyprogesterone acetate. The women in the progesterone group did not differ from those taking medroxyprogesterone acetate but they had somewhat lower values for high-density lipoprotein cholesterol and apolipoprotein AI than those receiving levonorgestrel.

The sequential addition of levonorgestrel significantly decreased high-density lipoprotein cholesterol and in particular the second subfraction of high-density lipoprotein cholesterol, whereas the third subfraction was unchanged. The decreases, which were apparent after one cycle, were about 18% and 28% ($p < 0.01$) for high-density lipoprotein cholesterol and the second

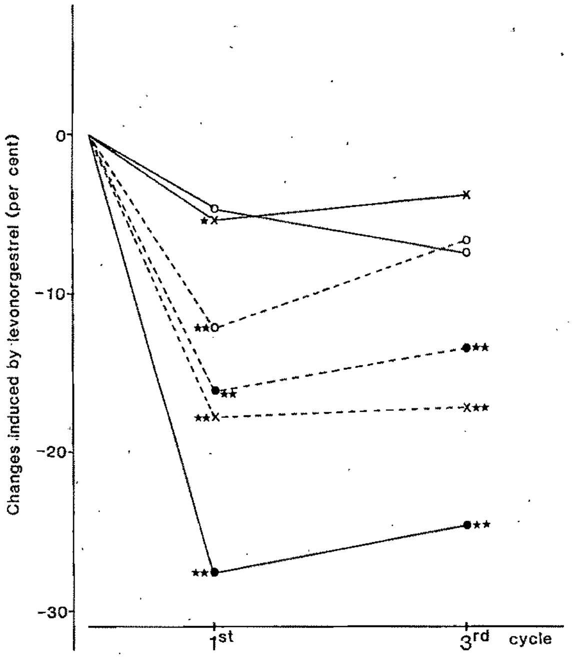

Fig. 1. Percentage of change in mean total cholesterol and high-density lipoprotein cholesterol, its subfractions, and apolipoproteins following the sequential addition of 250 µg of levonorgestrel daily in 20 estrogen-primed postmenopausal women. Key to symbols: Total cholesterol, x——x; high-density lipoprotein cholesterol, x- - -x; subfraction 2 of high-density lipoprotein cholesterol, ●——●; subfraction 3 of high-density lipoprotein cholesterol, o——o; apolipoprotein AI, ●- - -●; apolipoprotein AII, o- - -o. $*p < 0.05$; $**p < 0.01$.

subfraction of high-density lipoprotein cholesterol, respectively, and thereafter remained stable (Fig. 1). The same decline was found for apolipoprotein AI, whereas only weak transitory changes were recorded in apolipoprotein AII and total cholesterol. Significant effects on the lipoprotein pattern also were found after the administration of medroxyprogesterone acetate. Results were quite similar to those of the levonorgestrel group, in that the second subfraction of high-density lipoprotein and high-density lipoprotein cholesterol were the most sensitive parameters. The decrease was less pronounced but at the end of the third cycle high-density lipoprotein and subfraction 2 of high-density lipoprotein cholesterol were reduced by 8% and 17% ($p < 0.01$) (Fig. 2). The apolipoproteins were unchanged in this group. In women treated with micronized progesterone no significant changes in high-density lipoprotein cholesterol or any of its subfractions were observed (Fig. 3).

Comment

Apart from the protection against endometrial hyperproliferation, most other effects of progestogen addition during estrogen replacement therapy are basi-

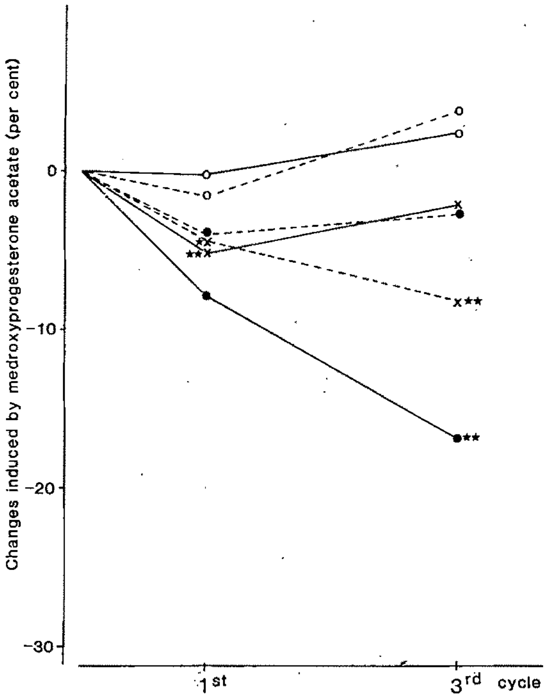

Fig. 2. Percentage of change in mean total cholesterol and high-density lipoprotein cholesterol, its subfractions, and apolipoproteins following the sequential addition of 5 mg of medroxyprogesterone acetate twice daily in 20 estrogen-primed postmenopausal women. For key to symbols, see Fig. 1.

cally unwanted and of little benefit to the patient. Therefore the choice of an appropriate progestogen is an important matter. Presently it is believed that there are clinically important differences between 19-norprogestogens and 17-hydroxyprogesterone derivatives.[5] Medroxyprogesterone acetate is often preferred because it has few androgenic side effects and is reported to have little or even a beneficial influence on blood lipids.[6-8]

While the present study confirms previous observations that levonorgestrel addition reduces high-density lipoprotein cholesterol in estrogen-primed postmenopausal women, it also demonstrates a similar effect of medroxyprogesterone acetate in a dose of 10 mg daily that is at variance with previous reports.[6-8] Hirvonen et al.[6] found the androgenic progestogens of the 19-nortestosterone series to reduce high-density lipoprotein cholesterol but concluded that medroxyprogesterone acetate had no such effect. Although the treatment regimen and the given doses of estrogen and medroxyprogesterone acetate were the same as in the present study, the clinical material consisted of only six postmenopausal women and indeed a slight but not statistically significant decrease of high-density lipoprotein cholesterol was present. Furthermore, other reports that medroxyprogesterone acetate will not interfere with lipoprotein metabolism have been based on comparatively few clinical observations, and often small but insignificant changes have been recorded.[7, 8] In our group of 20 postmenopausal women, at the end of the third treatment cycle, levels of high-density lipoprotein cholesterol and of subfraction 2 of high-density lipoprotein cholesterol were significantly reduced ($p < 0.01$). Though less pronounced, the pattern of the decline for the different lipoproteins was quite similar to that in women receiving levonorgestrel (Figs. 1 and 2). The high-density lipoprotein cholesterol consists of several subfractions. The protective role of high-density lipoprotein with regard to coronary heart disease has been attributed in particular to the second subfraction of high-density lipoprotein.[3] Among the various high-density lipoprotein components subfraction 2 was the most sensitive to progestogen addition, decreasing after 3 months by 17% and 25% for medroxyprogesterone acetate and levonorgestrel, respectively. Observations that parenteral administration of high doses of medroxyprogesterone acetate (for instance, during cancer treatment) only moderately decreases high-density lipoprotein cholesterol[13] have also been taken as evidence that the 17-hydroxyprogesterone derivatives are harmless compared to the 19-norsteroids. It should be recalled, however, that the route of administration is important in regard to the effects of steroid hormones on the liver. For example, oral estrogen therapy will increase the serum concentration of several estrogen-sensitive liver proteins, including high-density lipoprotein subfractions, in a dose-dependent manner, whereas there is no such effect when equipotent amounts of estrogen are given percutaneously.[14-16] Even high doses of estrogen given as intramuscular injections failed to produce increased amounts of the pregnancy zone protein, which is highly sensitive to oral estrogens.[17]

The present data suggest that doses and relative biologic activity of various progestogens are more important for their metabolic effects than are qualitative differences. On the analogy of previous reports on high-density lipoprotein cholesterol with the present study, levels of apolipoprotein AI, which is the major protein component of high-density lipoprotein, were found to be significantly depressed in women receiving 250 μg of levonorgestrel daily whereas there was only a slight and insignificant decrease after 10 mg of medroxyprogesterone acetate daily (Figs. 1 and 2). However, we do not think that it is justified to interpret this finding in terms of qualitative differences since there is little knowledge on the relative activity of the given doses. Comparative studies of the potency of various progestogens in different biologic systems are lacking. Adverse effects on blood lipids following administration of high doses of 19-nortestosterone derivatives such as 5 mg of norethindrone or 250 μg of levonorgestrel may reflect overtreatment rather than side effects especially related to these compounds. Indeed a low dose of norethindrone such as 1 mg, has little effect on high-density lipoprotein cholesterol[18] but still significant biochemical effects on the endometrium.[19] Recently megestrol acetate, also a 17-hydroxyprogesterone derivative, was found to decrease high-density lipoprotein cholesterol in climacteric women.[20] Thus the two groups of progestogens are probably equally suitable for sequential addition during estrogen replacement therapy.

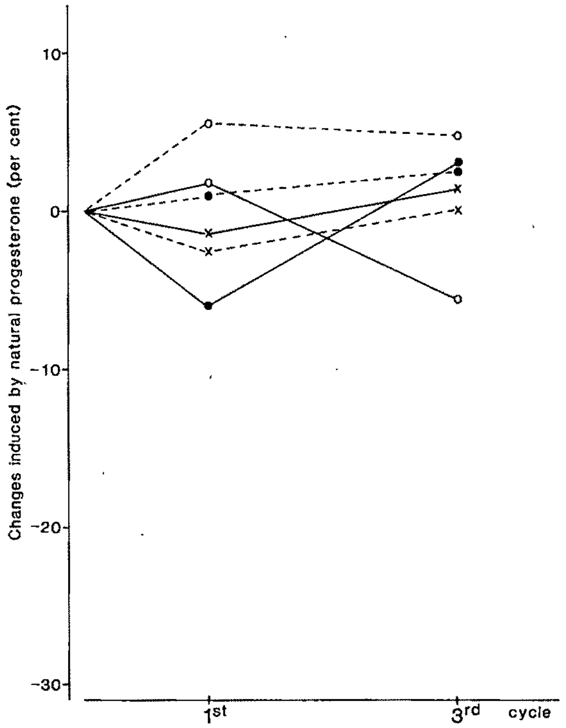

Fig. 3. Percentage of change in mean total cholesterol and high-density lipoprotein cholesterol, its subfractions, and apolipoproteins following the sequential addition of 100 mg of natural progesterone twice daily in 18 estrogen-primed postmenopausal women. For key to symbols, see Fig. 1.

Clinical treatment with natural progesterone has previously been hampered by the lack of an orally active preparation but, recently, significant absorption after oral administration of micronized progesterone has been demonstrated.[9] A single dose of 100 mg yields luteal phase values of circulating progesterone.[21] The present dose of 100 mg twice daily had no apparent influence on high-density lipoprotein cholesterol or apolipoproteins but was insufficient to provoke shedding in five of 17 women. Regular withdrawal bleeding occurred in 10 women and no clinical side effects such as weight gain or mood changes were reported. Recently 200 to 300 mg has been found to exert significant effects on the endometrium of postmenopausal women during estrogen replacement therapy.[22] It seems unlikely that a somewhat higher dose than given in the present study should adversely affect the serum lipoprotein pattern.

Micronized progesterone may develop into an attractive alternative to synthetic progestogens in clinical practice. Further studies on its therapeutic efficacy and possible side effects are necessary.

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Recurrent abortions, thromboses, and a circulating anticoagulant

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A syndrome occurring recently in young women and consisting of recurrent abortions, thromboses, and a circulating anticoagulant is described. It is suggested that an antiphospholipid antibody may be responsible for this clinical complex. (AM J OBSTET GYNECOL 1985;151:751-2.)

Key words: Abortion, thrombosis, anticoagulant, syndrome

In the past few years a novel syndrome has been recognized in certain young women. The illness includes recurrent abortions, thromboses, and (paradoxically) a circulating anticoagulant. We report a case of this syndrome and review recent studies suggesting that an antiphospholipid antibody may be responsible for the entire clinical complex.

Case report

A 26-year-old woman was admitted in November, 1983, with pain in the left upper thigh of 5 days' duration. Six weeks earlier, she had been delivered of a healthy baby. She had a history of four spontaneous first-trimester abortions, one in 1975, two in 1976, and one in 1980. In June, 1983, she received penicillin for a positive serologic test (VDRL) for syphilis. She had no history suggestive of systemic lupus erythematosus, jaundice, or prolonged or inappropriate bleeding.

Admission temperature was 38.9° C. There was a 10 cm area of tenderness over the left femoral triangle, and the circumference of the left thigh was 4 cm greater than that of the right. Hematocrit was 26%; leukocytes, 9600/μL with 67% neutrophils and 33% lymphocytes; and platelets, 440,000/μL. A direct Coombs test was positive. Antinuclear antibodies were detected at 1:5120, with a speckled pattern. Anti-DNA (native), anti–extractable nuclear antigen, anti–smooth muscle, and antiribonucleoprotein antibodies, and a lupus erythematosus cell preparation were all negative, as was a *Treponema pallidum* immobilization test.

Prothrombin time was 12.1 seconds (normal, 11.5); partial thromboplastin time, 50 seconds (normal 31 seconds); and Lee-White clotting time, 14 seconds (normal, 11 to 15 seconds). A reptilase time was normal. The factor II level was 98% of normal. Addition of an equal volume of normal plasma (mixing study) did not correct the prolonged partial thromboplastin time, and confirmation of the "lupus anticoagulant" was obtained by the thromboplastin inhibition test.

Heparin was given for the deep vein thrombosis which had been confirmed by blood pool venography, and glucocorticoids were given for persistent fever, painful thrombophlebitis, and possible hemolytic anemia. After 10 days of heparin, warfarin was started, glucocorticoids were rapidly tapered, and the patient was discharged on a regimen of warfarin in a dose sufficient to elevate the prothrombin time to approximately 2.5 times normal. Three weeks after discharge, the left thigh was no longer painful or tender.

Comment

Our patient had a false positive test for syphilis, an elevated titer of antinuclear antibodies, and a positive Coombs test, but she did not meet the criteria for a diagnosis of systemic lupus erythematosus. The salient feature of her case was the circulating anticoagulant.

An antithromboplastin type of circulating anticoagulant was first recognized in patients with systemic lupus erythematosus and was subsequently given the name "lupus anticoagulant." This term is somewhat misleading, since the same anticoagulant activity may be present in a variety of disorders. As many as half of all patients with circulating antithromboplastin anticoagulants do not appear to have systemic lupus erythematosus.[1] Moreover, a history of clinically significant bleeding is unusual in these patients, despite the prolonged partial thromboplastin time. Paradoxically, the incidence of thromboses appears to be high in patients who have an antithromboplastin anticoagulant. Inflammation and thrombosis of a leg vein, as in our patient, may occur. Thromboses of other veins or thromboses in the arterial circulation may also occur. A number of patients have had pulmonary emboli as well.

Fetal wastage is also correlated with the presence of the antithromboplastin anticoagulant. In their provoc-

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ative paper drawn from 20 years' experience at the National Center of Blood Transfusion in Paris, Soulier and Boffa[2] reported on three patients, each of whom had a circulating antithromboplastin anticoagulant and each of whom also had repetitive abortions (eight in Case 1, six in Case 2, five in Case 3) and thromboembolic accidents. Like our patient, none of these women met the standard diagnostic criteria for systemic lupus erythematosus, although fetal wastage is a well-known concomitant of that disorder.

Recent work has led to the suggestion that a common defect may be responsible for this spectrum of abnormalities. It has been suggested that the antibody responsible for antithromboplastin activity is directed against phospholipid epitopes and interferes with the "prothrombinase" complex (factor X_a, factor V, calcium, phospholipid). This or a similar antibody is also probably responsible for the biologic false positive test for syphilis, long known to be associated with systemic lupus erythematosus, and in this context the antibody has been called an anticardiolipin. Indeed, monoclonal anti-DNA antibodies derived from patients with SLE show polyspecificity and can bind to phospholipids as well as to polynucleotides. Antiphospholipid antibodies are also able to inhibit the production of prostacyclin, a potent blocker of platelet aggregation. Possibly by binding to phospholipid in the cell membrane, these antibodies prevent the release of arachidonic acid, a precursor in the biosynthesis of prostacyclin. Reduced production of prostacyclin would be expected to be associated with an increased frequency of thrombotic events.

Prospective studies should be undertaken to determine whether it is cost-effective to look for an antiphospholipid antibody in women with recurrent abortions and in patients with unexplained thromboses or a biologic false positive test for syphilis; a sensitive radioimmunoassay is now available for such screening. Administration of glucocorticoid and aspirin to six women with an antiphospholipid antibody and a history of intrauterine death in all 14 of their combined previous pregnancies resulted in suppression of the levels of the antibody in five patients. All five of these patients subsequently gave birth to live infants.[3] Similar therapy might benefit patients with recurrent thromboses.

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An unusual presentation of a case of staphylococcal pericarditis during pregnancy

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A case of a pregnant, drug-addicted patient with a pyogenic pericarditis without the systemic infectious manifestations is described. (AM J OBSTET GYNECOL 1985;151:752-3.)

Key words: Pregnancy, pericarditis

Acute pyogenic pericarditis is normally characterized by chest pain, fever, pericardial friction rub, and changes in the electrocardiogram. It is usually secondary to a septic focus somewhere else. Acute pyogenic pericarditis has been reported to present in a similar manner during pregnancy[1]; however, this condition can occur without all of these classical findings as shown by this unusual case.

Case report

L. H., a 30-year-old, gravida 8, para 6, abortus 1 woman, presented at 29 weeks' gestation by dates with a 3-day history of chest pain and mild shortness of breath. She denied fever and chills, although 2 weeks previously she had symptoms of an upper respiratory

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infection. The patient had a history of drug addiction and had been on methadone for the past 3 months. On physical examination, she was afebrile with a blood pressure of 120/70 mm Hg, a pulse of 94 bpm, and a respiratory rate of 18/min. The lungs were clear to auscultation. The heart had a regular rhythm with distant sounds, and no murmur was heard. Fresh needle marks were found on both forearms. The white blood cell count was $7200/mm^3$ with a normal differential count. The hematocrit was 31%. An electrocardiogram showed diffuse ST segment elevation, and a chest x-ray film revealed an enlarged heart shadow. Arterial blood gas values were pH of 7.49, Pco_2 of 26, and Po_2 of 102. Obstetric ultrasound showed a pregnancy at 25 weeks gestation. Although the findings were consistent with acute pericarditis, the patient refused hospital admission.

One week later she presented with increasing chest pain, shortness of breath, and leg edema. Physical examination was unchanged except for a heart rate of 104 bpm, pulsus paradoxus, and bilateral leg edema. At this time the hematocrit was 22.9%, and the white blood cell count was 12,000 with a normal differential count. An electrocardiogram showed a pericardial effusion but no valvular abnormalities. Liver enzymes (serum glutamic oxaloacetic transaminase, 197; serum glutamic pyruvic transaminase, 172) and serum haptoglobin levels (259, normal range = 27 to 139) were elevated. The cold agglutin titer was slightly elevated (1/16). Hepatitis B antigen and antinuclear antibodies were not present. The patient was diagnosed as having acute pericarditis. She was treated with indomethacin (25 mg three times daily) and furosemide (20 mg four times daily) and was given a 2 unit transfusion of packed red blood cells. She was discharged when her symptoms improved; however, she required a subsequent brief admission for chest pain.

At 29 weeks' gestation, by a reference ultrasound, she was admitted with increased shortness of breath, generalized edema, cool extremities, and a 9-pound weight gain within the previous week. She was afebrile, with a blood pressure of 104/90 mm Hg, a heart rate of 106 bpm, and a respiratory rate of 18/min. The lungs were clear. The jugular veins were distended, and the heart sounds were decreased. The fundal height was 33 cm; fetal heart sounds were present. Pitting edema was present in the lower extremities. An echocardiogram showed a persistent pericardial effusion but no valvular abnormalities.

A pericardiectomy was carried out and 500 ml of purulent pericardial fluid was removed. A drain was placed within the pericardium for postoperative drainage of pericardial fluid. Within a few hours of operation the patient went into labor and was delivered of a 29-week infant whose postnatal course was complicated by hyaline membrane disease, patent ductus arteriosus, and mild necrotizing enterocolitis. The mother developed a pleural effusion on the third postoperative day which required chest tube drainage. The placenta, pericardium, and pleural fluid grew *Staphylococcus aureus*. The patient was treated with intravenous sodium nafcillin for 12 days and then given vancomycin hydrochloride and rifampin for 30 days. The mother was discharged after 1½ months and the infant was discharged after 3 months. Both are doing well 3 months after discharge.

Comment

This case shows that severe acute pyogenic pericarditis can occur in a patient without fever or valvular abnormalities. This condition likely developed as a result of intravenous drug use. However, it cannot be determined whether the bacterial infection occurred primarily or developed subsequent to viral pericarditis. Nevertheless, a bacterial etiology must be considered in drug addicts with pericarditis even if they are afebrile.

Interestingly, the 4-week course of indomethacin therapy was not associated with pulmonary hypertension in the newborn infant; however, after delivery the patent ductus arteriosus responded to indomethacin therapy.

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Abruptio placentae following snake bite

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A case in which, besides the clinical complications that commonly follow snake biting, abruptio placentae occurred is reported here. None of the factors that are frequently associated with abruptio placentae were present. The laboratory tests performed showed that renal function was impaired and that a dramatic hypercoagulability was present. The relationship of the latter to the abruptio placentae is discussed. (AM J OBSTET GYNECOL 1985;151:754-5.)

Key words: Abruptio placentae, coagulation, snake bite

Snake venom poisoning in pregnant women is a peculiar complication. No medical study about the association of snake bite and pregnancy has been reported so far.

A case of a pregnant woman who was bitten by a snake of the genus *Bothrops* and developed abruptio placentae, coagulation alterations, and acute renal failure, is described here.

Case report

I. A. M. M., a 25-year-old white woman, gravida 2, para 1, came to our institution at 32 weeks of pregnancy complaining of genital hemorrhage of an hour's duration. She reported having been bitten by a snake, identified as *Bothrops jararaca*,* 6 hours before and had received 150 mg of antibothrops serum.

At physical examination she was in fair condition; she was pale (3+ to 4+) with a normal respiratory rate and without fever. The pulse was 80 bpm and the blood pressure was 100/80 mm Hg. Abdominal palpation showed uterine hypertonia. The uterine height was 32 cm and the abdominal circumference 92 cm. Fetal heartbeats were absent. At vaginal examination the cervix was dilated 2.5 cm dilatation, and the membranes were intact. The fetus was in a vertex presentation. There was moderate discharge of dark blood during the examination.

After the diagnosis of abruptio placentae, amniotomy was carried out and the amniotic fluid was bloody. Labor was induced with intravenous oxytocin. There was no progress in cervical dilatation, and a cesarean section was performed 6 hours later. A male stillborn infant weighing 2270 gm, with signs of recent death, was delivered. The placental weight was about 400 gm. Its insertion was corporeal, with 75% of the surface detached.

During the postoperative period the patient demonstrated marked oliguria, with increased plasma levels of urea and creatinine (Table I). Simultaneously, laboratory tests of the coagulation profile showed signs of disseminated intravascular coagulation (Table II). However, no clinical sign of coagulation disorder was observed at operation or during the postoperative period. The renal function was shown to be altered until the fifth postoperative day. The patient had a normal puerperal course and was discharged on the seventh postoperative day.

Comment

Snakes belonging to the genus *Bothrops* are responsible for most of the snake venom poisoning in Brazil. The venom of these snakes is characterized as proteolytic and coagulant in vivo and hemolytic in vitro.[1] Among the most serious complications of poisoning by *Bothrops* are disseminated intravascular coagulation and acute renal failure.[3] In this case, besides the above-mentioned clinical complications, the patient showed an obstetric one, abruptio placentae.

Although it is difficult to prove a direct involvement of *Bothrops* venom in the genesis of abruptio placentae, a relationship was observed in the present case. The mechanism which causes abruptio placentae is unknown, although there are some frequently associated factors.[2] Among these factors are arterial hypertension, compression of the inferior vena cava, trauma, uterine abnormalities, and a short umbilical cord. None of these conditions was observed in this patient.

The toxins contained in the venom of snakes of the genus *Bothrops* are activating agents of the coagulation systems; thus it can be suggested that such a complication may be related to the onset of abruptio placentae in this case. It has been shown that in cases of hypercoagulability there are fibrin deposition and formation

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Table I. Laboratory test results and urinary output

Day	*Hemoglobin (gm/dl)*	*Hematocrit (%)*	*Urea (mg/dl)*	*Creatinine (mg/dl)*	*Sodium (mEq/L)*	*Potassium (mEq/L)*	*Urinary output (ml/ 24 hr)*
1	11.7	37	56	1.7	136	5.8	410
2	11.4	35	65	2.9	133	6.7	3000
4	—	–	104	3.9	138	4.0	4000
5	7.2	24	82	2.7	—	—	—
6	6.8	23	58	1.8	137	4.2	1500
7	11.7	34	39	1.6	—	—	2000

Table II. Results of coagulation tests

Day	*Fibrinogen (mg/100 ml)*	*Prothrombin time (sec)*	*Recalcification time (sec)*	*Thrombin time (sec)*	*Activated partial thromboplastin time (sec)*	*Platelets (mm³)*	*Antithrombin III (%)*	*Fibrin degradation products (μg/ml)*
2	120 (165-485)	15 (15)	66 (65-140)	15 (15 ± 2)	47 (47 ± 4)	65.10^3 $(150\text{-}450 \times 10^3)$	62 (60-120)	16 (0-12)
3	250	14	74	15	44	42.10^3	105	10
9	390	14	97	15	40	165.10^3	97	4

Numbers in parentheses are normal values

of thrombus in the microcirculation of several organs. The eventual fibrin deposition and microthrombus formation at the spongy layer of the basal decidua could cause a cleavage and the consequent abruptio placentae.

In spite of abruptio placentae itself being a frequent cause of acute renal failure and of coagulation disorders, the participation of the *Bothrops* venom in causing impaired renal function and alteration of the coagulation system in this case cannot be excluded.

Since the patient came to our institution 6 hours after she had been bitten by the snake, it was not possible to establish whether the renal failure had occurred before the onset of the abruptio placentae. The experimental administration of the toxin contained in the venom of these snakes to pregnant animals may be an interesting means of demonstrating the pathogenetic mechanisms of abruptio placentae in this pathologic model.

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Lung hypoplasia and prolonged preterm ruptured membranes: A case report with implications for possible prenatal ultrasonic diagnosis

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A case of lung hypoplasia complicating prolonged preterm ruptured membranes is described, and the possible implications for prenatal ultrasonic diagnosis are discussed. (AM J OBSTET GYNECOL 1985;151:756-7.)

Key words: Fetal lung hypoplasia, premature rupture of membranes, ultrasound

Oligohydramnios as the cause of the nonrenal features of Potter's syndrome has been reported by Thomas and Smith.[1] Their "oligohydramnios tetrad of anomalies" included facial and ear anomalies, limb positioning defects, growth retardation, and, most important for the survival of the fetus, lung hypoplasia.[1]

With the trends toward observation and expectant management in preterm rupture of the membranes, the association of oligohydramnios and lung hypoplasia becomes increasingly important. The following case illustrates an infant with lung hypoplasia following prolonged rupture of membranes.

Case report

A 42-year-old, white woman, gravida 5, para 3-0-1-3, was referred to the Perinatal Section of Pennsylvania Hospital at 21 weeks' gestation because of preterm rupture of the membranes which occurred at 12 weeks' gestation during instrumentation. Despite knowledge of the poor prognosis for the fetus, the parents wished all measures for preservation of the pregnancy.

At the time of her first admission, the patient was found to have a fundal height consistent with 16 weeks' gestation. Fluid collected in the vaginal vault was positive for amniorrhexis by both fern and Nitrazine testing. Several ultrasound examinations were performed over the course of her pregnancy. These revealed marked oligohydramnios and increasing fetal compression with a decreasing ratio of thoracic circumference measured at the four-chambered view of the heart to abdominal circumference at the level of the umbilical vein (TC/AC) (Table I).[2]

At 25 weeks' gestation, she was readmitted because of vaginal bleeding and was treated with bed rest; she underwent frequent fetal monitoring. At 29 weeks, she demonstrated signs of abruptio placentae and fetal distress and underwent primary cesarean section. She was delivered of a 1355 gm living male infant with an Apgar score of 1 at both 1 and 5 minutes. Despite vigorous resuscitation, the infant died later on the morning of his birth.

Postmortem examination revealed Potter's facies, limb positioning defects, and pulmonary hypoplasia as evidenced by a combined lung weight of 18 gm (expected, 29 gm), and histologic evidence of poor development of the alveolar sacs beyond the alveolar ducts. Further examination revealed focal intra-alveolar and

Table I. Ultrasound examinations

	Date			
	1/28/83	*2/18/83*	*3/1/83*	*3/18/83*
Biparietal diameter	46	54	59	68
Weeks' gestation*	20.4	22.8	24.3	27.6
Thoracic circumference	15	16.5	19.0	18
Abdominal circumference	16	19	22	26
Head/abdomen ratio	1.19	1.13	1.07	1.0
TC/AC†	0.94	0.87	0.86	0.69

*Based on Yale tables of ultrasound criteria for gestational age according to biparietal diameter.
†Normal TC/AC, 0.94 (SD = 0.05).[2]

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interstitial hemorrhage, bilateral pneumothoraces, pulmonary interstitial emphysema, and mediastinal emphysema and hyaline membrane disease. A normal urinary tract including normal kidneys was found.

The mother's postoperative course was medically uncomplicated and she was discharged on the fifth postoperative day.

Comment

Preterm rupture of the membranes is managed expectantly in an effort to maximize the benefits of increasing fetal maturity while avoiding potential harm to the fetus and mother. Risks of this management scheme include maternal and fetal sepsis and, as our case illustrates, the additional risk of lung hypoplasia secondary to oligohydramnios and fetal thoracic compression.

Current management protocols for preterm ruptured membranes dictate delivery with evidence of maternal or fetal sepsis, yet the possibility of prenatal detection of lung hypoplasia has not been addressed. Our case illustrates a fetus born with lung hypoplasia and decreasing TC/AC.[2] A decrease in the TC/AC ratio as determined by ultrasound may be helpful in predicting lung hypoplasia secondary to oligohydramnios in the fetus, thus allowing the obstetrician to minimize this risk by an appropriately timed delivery.

Further study is needed to outline the pathophysiologic features of lung hypoplasia resulting from fetal thoracic compression and oligohydramnios and to define the role of ultrasound in its prenatal detection and management.

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Oxytocin augmentation of dysfunctional labor

V. An alternative oxytocin regimen

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A regimen is described in which the time from the start of a large dose of oxytocin to a myometrial response was used for selection of a maintenance dose. The method is based on our experience that continuous intravenous doses of oxytocin in the therapeutic range for the augmentation of labor require about 40 minutes of intravenous infusion to achieve a steady-state plasma concentration of oxytocin. The method effectively identified the proper maintenance dose on the first attempt in 43 of 59 patients (73%) and on the second in 58 of 59. The doses selected effected cervical dilatation in 55 of 59 cases (93%). Comparison of the timed dose method with an arithmetic progression regimen revealed the former method to select the minimum effective dose more rapidly, but that method had no advantage in shortening the time from the start of oxytocin to complete cervical dilatation. Examination of the plasma concentration of oxytocin by radioimmunoassay demonstrates that the maintenance dose selected by the duration of the interval from the start of infusion to a myometrial response sustains the plasma oxytocin concentration obtained by the initial dose. These data lend additional evidence that any particular dose of oxytocin in the therapeutic range requires approximately 40 minutes to achieve a steady-state plasma concentration. (AM J OBSTET GYNECOL 1985;151:757-61.)

Key words: Labor, oxytocin

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The continuous intravenous infusion of a specific dose of oxytocin produces a linear increment in the plasma concentration and a maximum steady-state value is reached in approximately 40 minutes.[1] Theoretically, ⅕ of the steady-state incremental value should be achieved in each successive 8-minute interval. This study was based on the assumption that the time from the initiation of a dose of oxytocin, which exceeded the needs of most patients with hypocontractility, to the time of a satisfactory myometrial response would identify the maintenance dose necessary to achieve cervical dilatation. Further, if the hypothesis were correct, the plasma concentration of oxytocin produced by the initial loading dose of oxytocin at the time of a satisfactory myometrial response should persist during the administration of the maintenance dose. It was our hope that this timed-dose method of administering oxytocin would shorten the time required from the initiation of oxytocin to full dilatation.

Material and methods

Patients. Sixty-five nulliparous patients in labor at term with arrest of cervical dilatation resulting from uterine hypocontractility composed the study group. There were six patients whose study was aborted by technical failure (plugged intrauterine pressure catheters, faulty oxytocin infusion system, computer malfunction), nine patients were delivered by cesarean section, and 50 were delivered vaginally. No patients were receiving continuous intravenous magnesium sulfate therapy or had intra-amniotic infection diagnosed at the time of initiation of oxytocin.

Oxytocin regimen. Each patient received an initial dose of 5 mU/min which was continued until the patient demonstrated a satisfactory response (see below) or 40 minutes elapsed without a response. If the uterus responded within the 40-minute period, a maintenance dose was selected according to the time from initiation of oxytocin to the first contraction of the myometrial response (first to ninth minute, 1 mU/min; tenth to seventeenth minute, 2 mU/min; eighteenth to twenty-fifth minute, 3 mU/min; twenty-sixth to thirty-third minute, 4 mU/min; thirty-fourth to fortieth minute, 5 mU/min). If the uterus did not respond in 40 minutes, the dose was increased to 10 mU/min and the maintenance dose selected by the time of response (forty-first to forty-eighth minute, 6 mU/min; forty-ninth to fifty-sixth minute, 7 mU/min).

Response recognition. The intrauterine pressure output of the labor monitor was interfaced with a bedside computer.[2] Within 20 seconds after the completion of each contraction, the computer printed a quantitative analysis: initial pressure, amplitude, period, and Montevideo units. Because the initial dose was in excess of the needs of a majority of patients it was necessary to minimize the number of contractions required for recognition of a response. Two consecutive contractions that were significantly different from the last six contractions preceding oxytocin therapy defined a response. A more precise definition cannot be provided because the pathophysiologic manifestations of hypocontractility vary from patient to patient. For example, 88% of the patients manifested periods of >200 seconds and 84% had <100 MU, but 50% had amplitudes ≥30 torr. The goal was to achieve two consecutive contractions with each manifesting >100 MU, amplitudes ≥30 torr, and periods ≤200 seconds. However, the response was dictated in part by the unique characteristics of the myometrium of each patient. A satisfactory response was accepted in a few patients with amplitudes of <30 torr but of sufficient frequency to produce in excess of 100 MU. In a few cases the uterus responded with less frequent but strong contractions.

Plasma oxytocin concentration. In a series of 10 patients, at the midpoint of the study, heparinized blood was drawn from an indwelling catheter in the following series: two samples 5 minutes apart before the start of oxytocin; one sample just prior to terminating the initial dose (when a satisfactory uterine response was recognized), and one sample every 15 minutes after the maintenance dose was initiated. Sample collection ceased if the cervix of the patient became fully dilated or after four samples were collected during the maintenance dose. Blood was placed in chilled glass tubes that contained heparin and were maintained on ice until centrifuged at 4° C. Plasma was frozen within 20 minutes of withdrawal and stored at −20° C until assay. Oxytocin was measured by radioimmunoassay after treatment of plasma with acetone and ether as previously reported.[3] All determinations were performed in duplicate in a single assay. The intra-assay coefficient of variation was <5% for all points of the standard curve.

Statistical methods. Forty-five nulliparous patients with hypocontractility and arrest of cervical dilatation who were treated with an oxytocin regimen consisting of arithmetic incrementations of the initial dose (1 mU/min) at intervals not <30 minutes[4] served as the control group. Student's two-tailed t test and χ^2 were used as appropriate.

Results

The method of selecting the maintenance dose from the duration of the interval from the initiation of oxytocin to a satisfactory response was effective on the first attempt in 43 of 59 cases (73%). In the remainder, the maintenance dose did not continue to obtain satisfactory contractility, and the dose selection procedure was

Table I. Comparison of timed-dose and arithmetic-dose regimens of oxytocin: vaginal deliveries

	Timed-dose group	*Arithmetic-dose group*	*p*
No. of patients	50	45	–
Dose (mU/min)			
At first cervical dilatation $>$1.0 cm	3.0 ± 2.1*	2.7 ± 1.4	NS
Maximum	3.5 ± 2.2	3.2 ± 1.7	NS
Time from start of oxytocin (min)			
To dose that produced cervical dilatation	37 ± 34	80 ± 96	$<$0.01
To maximum dose	66 ± 93	106 ± 110	0.05
To complete cervical dilatation	255 ± 189	292 ± 153	NS
Cervical dilatation at start of oxytocin (cm)	4.7 ± 1.3	4.1 ± 1.5	$<$0.05
No. of patients with oxytocin stopped or reduced	6	8	NS

*Mean ± SD.

repeated. In only one of 59 patients, three attempts were needed to identify the appropriate time-selected dose.

The maintenance dose selected effected $\geq$1.0 cm of cervical dilatation in 55 of 59 patients. In the 50 patients who were delivered vaginally the maintenance dose averaged 3.02 ± 2.09 (±SD) mU/min, and the dose the patient was receiving at the first notation of cervical dilatation was 2.96 ± 2.08 mU/min. In a minority of patients, cervical dilatation rates or the oscillographic contractile pattern did not meet the residents' expectations, and the dose was increased further. The maximum dose averaged 3.5 ± 2.2 mU/min.

The time from the initiation of oxytocin to the start of the dose that effected cervical dilatation $\geq$1.0 cm was 37 ± 34 minutes, and the time from the start to complete dilatation was 255 ± 189 minutes (Table I). If cervical dilatation was $<$5 cm (Table II) at the start of oxytocin therapy ($n = 27$ patients), the time to complete dilatation averaged 338 ± 204 minutes, and if $\geq$5 cm ($n = 23$ patients), 158 ± 118 minutes ($p < 0.01$). Of the patients who were delivered vaginally, 49 of 50 required $\leq$10.5 hours to complete cervical dilatation, and 44 of 50, $\leq$8 hours. The oxytocin therapy was stopped or the dose reduced in six cases because of hypercontractility, abnormalities of the fetal heart rate, or both of these indications. One patient manifested hypercontractility during the first few minutes of the initial infusion.

The plasma concentration of oxytocin prior to treatment of the 10 patients was 0.83 ± 0.12 μU/ml (mean ± SEM). This value is similar to that reported by Leake et al.[5] and ourselves.[1] The plasma concentration of oxytocin rose to an average of 1.58 ± 0.18 μU/ml at the time a significant uterine response was identified and did not change significantly at 15 or 30 minutes from the start of the maintenance dose (Fig. 1). In four patients full dilatation was reached before the 45-minute samples could be drawn. In the remaining six patients, the 45- and 60-minute samples were obtained and demonstrated mean values (1.77 ± 0.32 and 1.70 ± 0.39 μU/ml) almost identical to that observed at the termination of the initial dose (1.65 ± 0.25). At 45 minutes after the maintenance dose was initiated, the mean increment in the plasma concentration of oxytocin was 1.03 μU/ml with a mean dose of 1.83 mU/min. In these six women, with a mean weight of 65.4 kg, the plasma clearance rate was 27.2 ml/kg/min.

Table II. Comparison of timed-dose group versus arithmetic-dose group

Cervical dilatation prior to oxytocin (cm)	*Time from start of oxytocin to complete dilatation (min)*	
	Timed-dose group	*Adjusted-dose group*
$<$5	316 ± 172 (27*)	348 ± 129 (28)
$\geq$5	158 ± 119 (23)	200 ± 149 (17)

*Number in parentheses is number of patients.

Comparison of the results obtained with a timed-dose method of selection (50 patients) and those obtained with an arithmetic incremental dose regimen (45 patients) demonstrated no difference in the mean dose required to effect at least 1 cm of dilatation or in the maximum dose (Table I). The timed-dose group demonstrated a shorter mean time from the start of oxytocin to the start of the dose that effected cervical dilatation ($p < 0.01$) and to the maximum dose ($p = 0.05$) but had greater initial cervical dilatation ($p < 0.05$). These advantages did not result in any significant difference in the mean time required from the initiation of oxytocin therapy to complete dilatation (255 ± 189 versus 292 ± 153 minutes), or in the frequency of stopping the treatment or reducing the dose because of concerns with hypercontractility or abnormalities of the fetal heart rate.

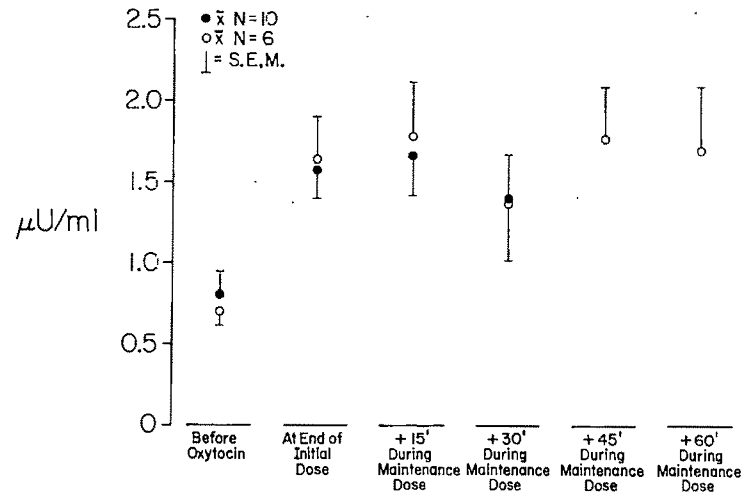

Fig. 1. The plasma oxytocin concentration before the administration of oxytocin, at the end of the initial dose when a significant myometrial response was identified, and at 15-minute intervals after the start of the maintenance dose.

Comment

The timed-dose method relies upon the assumption that the infusion of a continuous therapeutic dose of oxytocin requires approximately 40 minutes to produce a steady-state plasma oxytocin concentration; this has been confirmed by direct measurement during arithmetic dose increments.[1] The present study reinforces this conclusion in two ways: It demonstrates that a maintenance dose selected on the basis of the time to uterine response of an infusion of oxytocin greater than required can maintain both the plasma oxytocin concentration and the myometrial response.

The common belief that oxytocin has a short half-life and that infusion of a singular dose produces a steady-state plasma concentration in 20 minutes[6] are based on three types of experiments. First, the addition of oxytocin to human pregnancy plasma results in rapid destruction by the cystine-aminopeptidase of placental origin. Most investigators observe a half-life of 5 to 10 minutes in vitro.[7] Second, the administration of quantities of oxytocin (500 to 16,000 mU) sufficient to provide plasma levels detectable by bioassay has resulted in half-life values <5 minutes.[8, 9] Third, studies of the administration of tritium-labeled oxytocin to pregnant women also identified a mean half-life of <5 minutes.[10] However, 5000 mU of tritium-labeled oxytocin was injected as a bolus for these studies.

The contrary evidence comprises both physiologic and biochemical observations. Sica-Blanco and Sala[11] infused doses of oxytocin in the "pharmacologic range" of 1 to 16 mU/min to pregnant women at term for 1 hour and noted that the time required to achieve a steady-state myometrial response ranged from 14 to 60 minutes. At very high rates of infusion (32 mU/min), this interval was usually "shorter" and at 8000 mU/min it was extremely brief with the production of a pathologic resting pressure in 1 minute. When the dose of intravenously infused oxytocin is the minimum required to effect a response, the time from initiation of oxytocin to the desired pharmacologic response will approximate 40 minutes. As the dose is incremented above this therapeutic quantity, the time to response will be shorter because the minimum plasma concentration of oxytocin necessary to effect a result will be achieved more quickly. There is no evidence of any significant lag time between the advent of an effective plasma concentration of oxytocin and myometrial response, as the administration of much larger doses than necessary evokes a myometrial response as quickly as 1.2 minutes after the start of the infusion.[12]

Leake et al.[5] infused 4 mU/min into women 37 weeks pregnant until a steady-state plasma level was obtained and calculated the plasma clearance rate. The mean plasma clearance rate obtained in these women was 23.1 ml/kg/min, not significantly different from that observed in men and nonpregnant women. We have confirmed their observations of a similar plasma clearance rate of oxytocin during hypocontractile labor.[1] Further, when our patients received 1 to 8 mU/min, we could find no difference in the plasma concentration of oxytocin by 40, 60, and 80 minutes after the start of the minimum effective dose, indicating that the continuous infusion of the minimum effective dose produces a steady-state plasma concentration of oxytocin by 40 minutes.

Some serious limits of the study of oxytocin pharmacokinetics prior to the advent of radioimmunoassay can be identified. The use of large doses of oxytocin in the bioassay studies may have exposed the oxytocin to enzymes which are substrate limited and are not operative at the usual plasma concentrations found in common therapeutic situations. The same complaint is valid for the studies of the disappearance rate of tritium-labeled oxytocin from plasma as well. Further, the rate of catabolism of oxytocin dissolved in pregnancy plasma is no certain measure of in vivo events. Each study of oxytocin metabolism by means of radioimmunoassay has demonstrated that a continuous intravenous infusion of a dose of oxytocin in the pharmacologic range produces a linear increase in the plasma concentration of oxytocin which reaches a steady-state value in about 40 minutes, and the plasma clearance rates of men and of pregnant and nonpregnant women are similar.

Our clinical studies in this and a prior report[2] total 113 nulliparous patients treated with oxytocin regimens on the basis of the presumption that any single dose of oxytocin produces a slow steady rise in the plasma concentration of oxytocin to steady-state value in about 40 minutes. Of the patients who received doses in the range of 1 to 9 mU/min, 84% were delivered vaginally and 16% by cesarean section. Of the patients who were delivered vaginally, 94 of 95 required <10.5 hours of oxytocin treatment to achieve complete dilatation and 86 of 95, <8 hours. Associated pathologic states were found in 17 of 18 patients who underwent cesarean section: intra-amniotic infection, seven cases, two with macrosomia; occipitoposterior position, five cases; contracted pelvis, three cases; macrosomia alone, 1 case; and failed descent with attempted vacuum extraction, one case. One of the 18 patients had no demonstrable abnormality and may have benefited from a larger dose of oxytocin than the maximum of 3 mU/min which she received. Our experience suggests that larger doses would not significantly reduce the rate of cesarean section.

These studies demonstrate that shortening the time from initiation of oxytocin to the ascertainment of the necessary dose to effect cervical dilatation is not a significant factor in determining the time necessary to achieve complete dilatation. The timed-dose method does not provide any special clinical advantage and has the deficit of requiring a constant bedside professional attendant until the proper dose is identified. This study demonstrates that the forces of resistance in the cervix, not the rapidity of identification of the proper oxytocin dose, are the major determinant of the time required to produce complete dilatation.

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Cardiovascular alterations in severe pregnancy-induced hypertension: Relationship of central venous pressure to pulmonary capillary wedge pressure

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The relationship between central venous pressure and pulmonary capillary wedge pressure was studied in 18 patients with severe pregnancy-induced hypertension. Although statistically a linear relationship for the group as a whole could be identified, analysis on a case-by-case basis revealed different results. In 10 patients, a linear correlation between central venous pressure and pulmonary capillary wedge pressure was observed. However, accurate prediction of pulmonary capillary wedge pressure from central venous pressure was not possible even in this group because of large interindividual variations. In seven patients no correlation between central venous pressure and pulmonary capillary wedge pressure could be identified. In the last patient a curvilinear relationship existed between central venous pressure and pulmonary capillary wedge pressure. Additionally, in five cases of pulmonary edema, a negative gradient of colloid osmotic pressure to pulmonary capillary wedge pressure gradient was observed. Our data suggest that central venous pressure is not a clinically reliable predictor of pulmonary capillary wedge pressure. (AM J OBSTET GYNECOL 1985;151:762-4.)

Key words: Pregnancy-induced hypertension, vascular changes, pulmonary edema

The use of cardiovascular hemodynamic monitoring has assumed an increasing role in the care of the critically ill obstetric patient. Previous investigations[1-3] with the use of hemodynamic monitoring in patients with severe pregnancy-induced hypertension have suggested a disparity between right and left ventricular function in this disease process. The present study was undertaken to determine the relationship between central venous pressure and pulmonary capillary wedge pressure when obtained via a pulmonary artery flow-directed catheter in patients with severe pregnancy-induced hypertension.

Material and methods

Eighteen patients with severe pregnancy-induced hypertension, as defined by the American College of Obstetricians and Gynecologists, undergoing central hemodynamic monitoring with a pulmonary artery flow-directed catheter made up the study group. After written informed consent was obtained, a pulmonary artery flow-directed catheter was inserted percutaneously via either the internal jugular or the subclavian vein and guided into the pulmonary capillary wedge position with the use of continuous pressure monitoring.

The criteria for verifying a true wedge position included: (1) the characteristic waveform, (2) the mean pressure lower than the mean pulmonary arterial pressure, (3) the presence of respiratory variation, and (4) roentgenographic confirmation of placement in the pulmonary artery. Measurements were made with the patient supine and, if prior to birth, a wedge under the right hip. Repeated measurements were made in individual patients at various filling pressures in order to evaluate the response of pulmonary capillary wedge pressure to changes in central venous or right atrial pressure. No attempt was made to exclude patients because of previous drugs or clinical condition. Regression analysis was used to determine if either a linear or a quadratic relationship existed between central venous pressure and pulmonary capillary wedge pressure.

Results

Two hundred seven separate simultaneous determinations of central venous pressure and pulmonary capillary wedge pressure were made in 18 patients. The number of determinations per patient ranged from four to 27. The lowest central venous pressure recorded for any one patient was −1 mm Hg and the

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Table I. Distribution of pulmonary capillary wedge pressures for each observed central venous pressure

Central venous pressure (mm Hg)	*Pulmonary capillary wedge pressure (mm Hg)*		
	Mean ± SD	*Minimum*	*Maximum*
−1	5.0	5	5
0	5.2 ± 0.9	4	6
1	5.4 ± 2.3	3	11
2	7.4 ± 4.4	4	17
3	7.8 ± 1.9	5	12
4	8.3 ± 1.6	5	12
5	8.8 ± 2.5	5	13
6	10.7 ± 3.0	6	18
7	9.9 ± 2.1	7	13
8	12.7 ± 3.5	3	21
9	13.3 ± 3.5	9	17
10	15.0 ± 3.4	10	22
11	15.8 ± 4.0	12	22
12	18.3 ± 4.2	15	23
13	15.6 ± 2.3	13	17

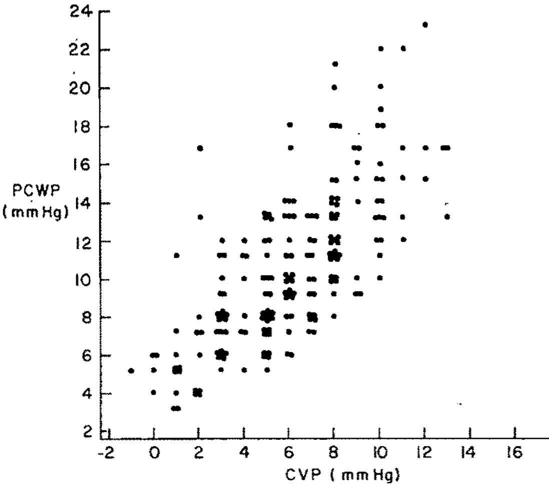

Fig. 1. The relationship of central venous pressure *(CVP)* to pulmonary capillary wedge pressure *(PCWP)* in 18 patients with severe pregnancy-induced hypertension is shown.

lowest pulmonary capillary wedge pressure was 5 mm Hg. The highest central venous pressure recorded was 13 mm Hg and the highest pulmonary capillary wedge pressure was 23 mm Hg. In Fig. 1 the relationship of central venous pressure to pulmonary capillary wedge pressure for the group as a whole is shown. It can be seen that overall a positive linear relationship appears to exist between central venous pressure and pulmonary capillary wedge pressure. However, since there were several measurements on each individual patient, the measurements for one individual are related to each other in a different manner rather than to measurements from a different patient. This problem was dealt with by performing a regression analysis separately on the data from each patient. Analysis of each individual case revealed that a linear relationship could actually be demonstrated in only 10 patients. In seven cases (39%) no linear or quadratic relationship could be shown between central venous pressure and pulmonary capillary wedge pressure. In one case the data were best fit by a quadratic equation.

It can also be seen in Fig. 1 that substantial variations occurred in terms of the pulmonary capillary wedge pressure for a given central venous pressure. In clinical terms, any given central venous pressure was associated with an unacceptably wide range of pulmonary capillary wedge pressures. In Table I, the mean (±SD) and range of pulmonary capillary wedge pressures for different central venous pressures are given.

For the case in which a quadratic relationship was identified, small changes in central venous pressure resulted in large increases in pulmonary capillary wedge pressure. In that patient, 27 separate determinations were analyzed. The largest difference observed in that patient was 10 mm Hg with a central venous pressure of 8 mm Hg and a pulmonary capillary wedge pressure of 18 mm Hg. The largest difference between central venous pressure and pulmonary capillary wedge pressure observed in the overall study group was 15 mm Hg. This occurred in a patient in whom a linear relationship did not exist. In that case the central venous pressure was 2 mm Hg and the pulmonary capillary wedge pressure was 17 mm Hg.

Five patients in the study group developed clinical pulmonary edema. In four of the five patients a linear relationship between central venous pressure and pulmonary capillary wedge pressure was demonstrated. One patient showed no demonstrable correlation. The lowest central venous pressure observed among the patients with clinical pulmonary edema was 9 mm Hg and the lowest pulmonary capillary wedge pressure was 14 mm Hg. Analysis of the colloid osmotic pressure to pulmonary capillary wedge pressure gradient invariably revealed a negative relationship. The relationship of colloid osmotic pressure to pulmonary capillary wedge pressure for patients with pulmonary edema is shown in Table II.

Comment

In the absence of cardiopulmonary dysfunction, central venous pressure has been accepted as an adequate guideline for managing shock and hypovolemia.[4-6] However, a growing consensus is developing that in seriously ill patients, right and left ventricular function are so disparate that not only the absolute central venous pressure but even central venous pressure changes are unreliable and misleading estimates of left heart filling.[7-14]

Table II. Relationship of colloid osmotic pressure to pulmonary capillary wedge pressure in pulmonary edema

Patient No.	*COP (mm Hg)*	*PCWP (mm Hg)*	*Gradient (mm Hg)*
1	20.7	23	−2.3
2	11.7	14	−2.3
3	14.3	17	−2.7
4	13.1	17	−3.9
5	15.0	17	−2.0

PCWP = Pulmonary capillary wedge pressure. COP = Colloid osmotic pressure.

In this study of patients with severe pregnancy-induced hypertension, a linear relationship between central venous and pulmonary capillary wedge pressure was identified in 56% of cases. However, accurate prediction of the pulmonary capillary wedge pressure from central venous pressure was precluded even in this group because of the wide interindividual variations seen. The fact that no relationship between central venous pressure and pulmonary capillary wedge pressure could be recognized in 39% of patients provides additional support for the use of the pulmonary artery flow-directed catheter in critically ill patients with severe pregnancy-induced hypertension.

Pulmonary edema in patients with pregnancy-induced hypertension has previously been attributed to a decreased gradient of colloid osmotic pressure to pulmonary capillary wedge pressure.[15, 16] Our data showing a negative gradient of colloid osmotic pressure to pulmonary capillary wedge pressure in all five patients with pulmonary edema support this concept. Benedetti and Carlson[17] reported a mean postpartum colloid osmotic pressure of 13.7 mm Hg in 22 patients with pregnancy-induced hypertension. That information coupled with the report of Henderson et al.[16] of a postpartum mean pulmonary capillary wedge pressure of 13 mm Hg in patients with pregnancy-induced hypertension suggests that this group of patients is at substantial risk for disruption of the normal relationship of colloid osmotic pressure to pulmonary capillary wedge pressure.[18]

In summary, our study identified a linear relationship between central venous and pulmonary capillary wedge pressure in 10 of 18 (56%) patients with severe pregnancy-induced hypertension. Even in patients in whom a linear relationship existed, large individual-to-individual differences in gradients of central venous pressure to pulmonary capillary wedge pressure prevented accurate prediction of pulmonary capillary wedge pressure from central venous pressure. Finally, in our study, clinical pulmonary edema was uniformly associated with a negative gradient of colloid osmotic pressure to pulmonary capillary wedge pressure.

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Chlamydial endocervical infections and cytologic findings in sexually active female adolescents

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The association of infection with *Chlamydia trachomatis* and cytologic changes on Papanicolaou smear was examined in 148 sexually active postmenarchal female subjects, aged 13 to 21 years (mean = 17.2) attending a teen clinic. Endocervical samples for micro-organisms (*C. trachomatis* and *Neisseria gonorrhoeae*) and a cervical sample for cytologic examination were taken. A detailed evaluation of the cytologic results was made independently of the *C. trachomatis* status. In 23 (15.5%) subjects tests for isolation of *C. trachomatis* were positive. Inflammatory changes in epithelial cells, nuclear changes in metaplastic cells, and lymphocytes in the inflammatory exudate were associated with *C. trachomatis* isolation but suspected "chlamydial inclusions" and cytoplasmic vacuoles in metaplastic cells were not. The results reported here do not support the use of cervical cytologic examination as a definitive diagnostic test for presence of an endocervical chlamydial infection. However, it may be possible to use the cytologic pattern described here to identify a population with a high prevalence of *C. trachomatis*. (AM J OBSTET GYNECOL 1985;151:765-71.)

Key words: *Chlamydia trachomatis*, culture, Papanicolaou smear, diagnosis

Chlamydia trachomatis is a common sexually transmitted disease organism in sexually active female adults and adolescents.[1-3] Laboratory diagnostic tests for chlamydia include direct microbiologic isolation and serologic and cytologic techniques. Direct isolation, considered the most sensitive and specific test, requires sophisticated cell culture techniques that are unavailable in most clinical laboratories. The serologic microimmunofluorescent procedure is highly sensitive and specific for chlamydia but usually does not enable distinguishing a current from a past infection. Cytologic staining techniques[4] have played an important role in defining the diagnosis of chlamydial conjunctivitis in the newborn infant. Iodine is the least sensitive of the three methods for identifying chlamydia in tissues and is not used with cervical specimens as they normally include glycogen-containing cells. Giemsa, the traditional stain for chlamydial identification on cytologic specimens, is highly sensitive for identifying chlamydial conjunctivitis in the newborn infant; it is, however, insensitive for identifying genital tract infection.

The value of the Papanicolaou-stained cervical smear for the detection of *C. trachomatis* infections is controversial. Several authors[5, 6] have described chlamydia-associated cellular changes in Papanicolaou-stained materials. Cells infected by chlamydia have been described as large (15 to 20 μm or larger), "atypical" metaplastic cells or as endocervical columnar cells with vacuolated cytoplasm that contain inclusion bodies at three stages of development. Other investigators,[7] using Papanicolaou staining, have not found intracytoplasmic inclusion bodies to be pathognomonic for chlamydial infection, although they found that inflammatory changes had increased in cells.

We studied Papanicolaou-stained smears from sexually active female adolescents to determine the value of cytologic testing for the detection of *C. trachomatis* infection and to investigate whether particular intracytoplasmic inclusion bodies are pathognomonic for *C. trachomatis* infection.

Methods and material

Informed consent was obtained from all patients according to the guidelines of the Committee on Human Research, University of California (San Francisco).

Participants. One hundred forty-eight sexually ac-

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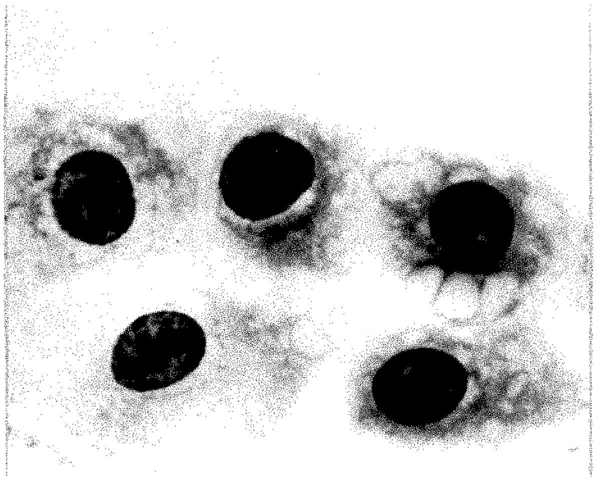

Fig. 1. Cytoplasmic vacuolization and nuclear changes in metaplastic cells from a *C. trachomatis*–positive patient. (× 400.)

tive female adolescents aged 13 to 21 years (mean = 17.2 years) presenting at the Teen Clinic of the University of California (San Francisco) were examined. The participants had not taken antibiotics, had no spontaneous or therapeutic abortions within 30 days, were not acutely ill with a nongenitourinary infection, and were not menstruating on the day of examination.

Genitourinary history and examination. Participants completed a questionnaire on routine sexual and genitourinary history and socioeconomic status information.[8] A genitourinary history and examination were obtained by the clinician (adolescent medicine fellow or faculty) and recorded on standardized forms. Examinations were supervised by one author (M. A. S.).

Microbiology. Endocervical cultures for *C. trachomatis* were obtained from the 148 participants. For control purposes, endocervical cultures for *Neisseria gonorrhoeae* and Papanicolaou smear evaluation for both yeast and *Trichomonas vaginalis* were also performed on the 148 participants. Seventy-nine of the 148 participants had additional endocervical cultures obtained for *Mycoplasma* species and *Ureaplasma urealyticum.*

Methods

A sterile, nonlubricated speculum was inserted into the vagina and cytologic specimens were obtained. Cultures of the endocervix were then obtained with the use of Dacron swabs and immediately inoculated onto modified Thayer-Martin agar *(N. gonorrhoeae),* A7B agar, and U9B broth (*Myocoplasma* species and *U. urealyticum*).[9, 10] An applicator was then used to scrape cells from the endocervix and to inoculate cells directly into modified Eagle's medium broth *(C. trachomatis).* The applicator was removed from the broth before transportation to the laboratory where it was processed for *C. trachomatis* as described previously.[11]

Collection of specimens. Cervical-vaginal samples were collected from the 148 women. A vaginal scraping, a pancervical scraping, and an endocervical aspirate were collected sequentially and spread on three separate slides for 73 women. In the remaining 75 women, samples included a pancervical scraping and an endocervical scraping, or an aspirate only.

The slides were immediately fixed in 95% ethanol, stained by a modified Papanicolaou technique, mounted with Permount (Fischer Scientific Co.), and microscopically evaluated, and the diagnosis was recorded. Each slide was reevaluated by two research cyto-technologists, without knowledge of the reported cytologic diagnosis or chlamydial culture results, for general specimen quality, hormonal pattern, presence of endocervical and endometrial cells, epithelial cell changes, and microorganisms. A qualitative assessment of epithelial and nonepithelial cells was made; metaplastic cells were considered "rare" if less than five single cells or groups of cells were found, "few" if five to 10, "moderate" if 11 to 25, and "predominant" if more than 25 cells were present. The same system was used to quantify metaplastic cells with cytoplasmic vacuoles or those with nuclear change. The nonepithelial cells were quantified by assessing five separate high-power fields (× 400 objective) with the largest number of cells of a given type. "Rare" referred to one to four cells in five fields, "few" to five to 10 cells, "moderate" to 11 to 100, and "predominant" for more than 100 cells. For lymphocytes and plasma cells, the assessment was made on only one high-power field. Particular attention was given to the presence of metaplastic cells, nuclear change in cytoplasmic vacuoles, and densities interpreted as inclusions within vacuoles in these cells. All cases were reviewed by one pathologist (E. B. K.) and the results of the detailed screening were systematically coded.

Classification of cells. The features of inflammatory epithelial cells (cytologic inflammation), which varied from normal cell morphologic characteristics, were increased cell size, altered cytoplasmic staining reaction, vacuolization in squamous cells, and perinuclear halos. Nuclear changes associated with an inflammatory reaction were multinucleation, increased size, hyperchromasia, coarse chromatin granularity, or nucleolar prominence. Inflammatory cells were quantified separately and were not included in the definition of cytologic inflammation. Metaplastic cells were arbitrarily defined as immature squamous cells having a parabasal-like maturation. This definition of metaplasia in cytologic samples is widely accepted, but proof of metaplasia depends on the examination of tissue biopsy specimens and the recognition of replacement of columnar by squamous epithelium in cervical mucosal tissue. The cytologic diagnosis was described in the mor-

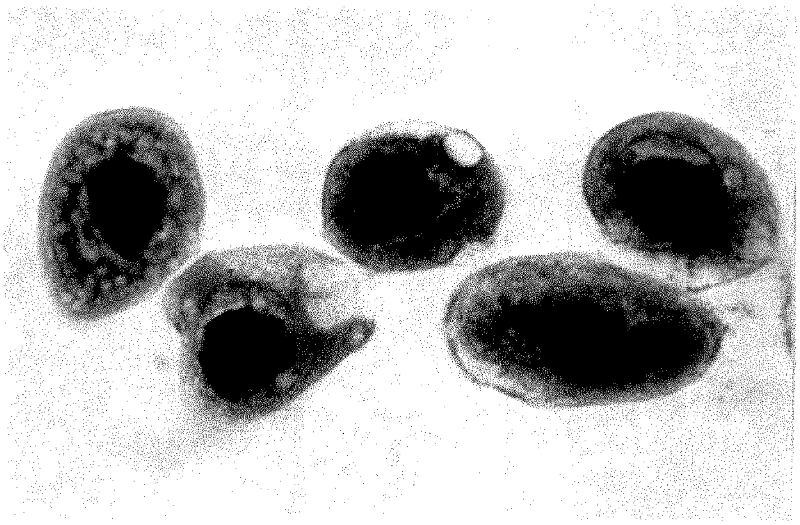

Fig. 2. Cytoplasmic vacuolization and nuclear changes in metaplastic cells from a *C. trachomatis*–negative patient. (×400.)

phologic terms used for histologic diagnosis: benign (no significant cellular abnormalities), dysplasia (mild, moderate, or severe), or malignant (cell type and origin). Koilocytosis was identified as a very prominent perinuclear clearing bordered by a condensed ectoplasmic rim associated with condyloma and dysplasia.[12] Repair referred to specific cytomorphic features in squamous, columnar, or undifferentiated cells that have been shown to be related to histologic evidence of repair. Only those changes with atypical repair cells[13] were included.

After frequencies of cytologic findings were determined, the association of *C. trachomatis* with specific cytologic findings was evaluated by the χ^2 test.

Results

Sociodemographics. The mean menarchal age of subjects was 12.2 years (r = 8 to 15 years). Fifty-five (44%) were black, 46 (37%) were white, 14 (11%) were Hispanic, 10 (8%) were Asian or other, and for 23 racial information was not recorded. The socioeconomic status indicated that the typical paternal occupation was skilled manual work and the average maternal education was 2 years of college; these findings did not differ significantly among racial groups. Forty-four (32%) were oral contraceptive users, 36 (26%) barrier users (mainly condoms), and 57 (42%) used withdrawal or no contraceptives; contraceptive information was not recorded for 11.

Microbiologic features. *C. trachomatis* was isolated from the endocervices of 23 of the 148 participants (15.5%). In addition, *N. gonorrhoeae* was isolated from seven of 140 (5%) subjects; *Mycoplasma* species was isolated from 36 of 79 (46%) subjects tested; *U. urealyticum* was isolated from 59 of 79 (75%) of subjects tested.

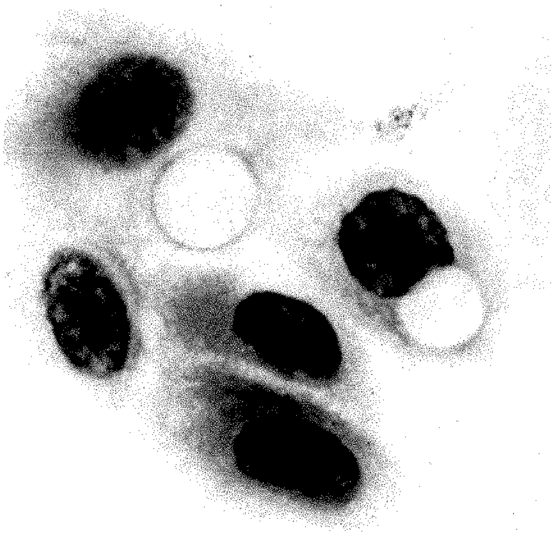

Fig. 3. Larger discrete vacuoles in metaplastic cells from a *C. trachomatis*–positive patient. (×400.)

Trichomonads were identified on Papanicolaou smears from 19 of the 148 subjects (12.8%), and yeast in 21 of 148 (14.2%) subjects.

Cytologic features. The cytologic diagnosis was benign in 98% (145 of 148) of the participants. Two instances of mild dysplasia and one of moderate dysplasia were found among chlamydia-negative women only. Epithelial changes interpreted as inflammatory were noted in 65 (43.9%) women. Koilocytosis was not identified; repair was found in a single specimen. Endocervical cells were absent in 22 (14.9%) women; they were, however, absent in only one of the 23 *C. trachomatis*–positive cases. Moderate to many metaplastic cells were noted in 57 (38.5%) women. Neutrophils and his-

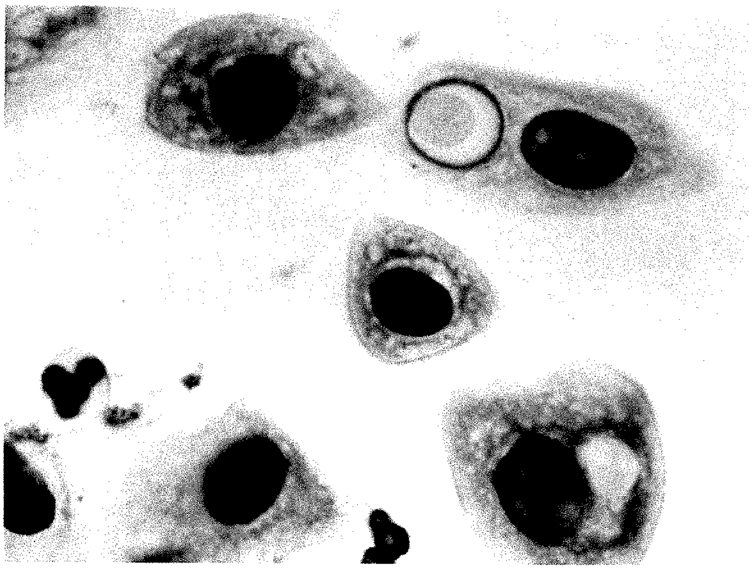

Fig. 4. Larger discrete vacuoles in cells from a *C. trachomatis*–negative patient. (×400.)

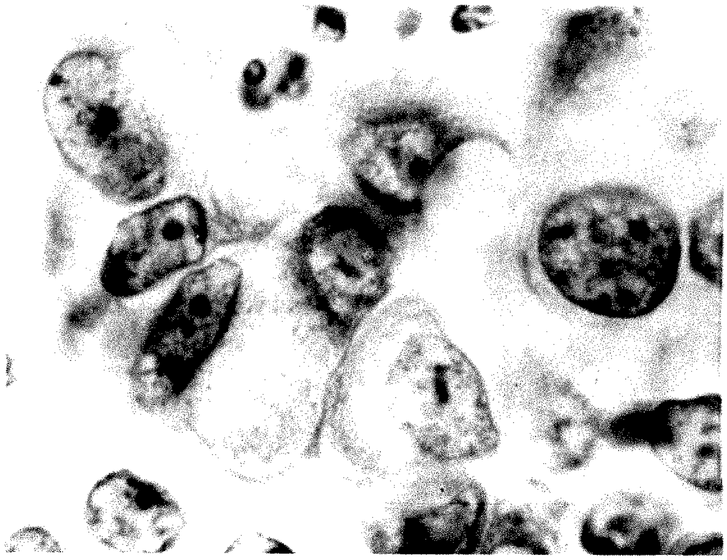

Fig. 5. Fine granular densities or "inclusions" in cells from a *C. trachomatis*–positive patient. (×400.)

tiocytes were frequent findings; moderate to many neutrophils were seen in 147 (99.3%) women, and histiocytes in 140 (94.6%) women (Figs. 1 to 7).

Analysis. In comparing the cytologic findings to the results of chlamydial isolation, three findings were significantly associated with chlamydia initially: cytologic inflammation, a moderate number of lymphocytes, and a moderate number of metaplastic cells having nuclear changes (Table I). These associations remained significant when *N. gonorrhoeae*, *T. vaginalis*, yeast, and viral inclusions were controlled for separately. Neither vacuoles in metaplastic cells nor suspected "chlamydial inclusions" were associated with chlamydia.

Cytologic inflammation, lymphocytes, and nuclear changes in metaplastic cells were entered into a stepwise logistic regression analysis to determine their joint association with chlamydial infection. Nuclear changes in metaplastic cells did not meet the 0.05 significance level for inclusion in the model, and this parameter was removed from the analysis. The combination of cytologic inflammation and lymphocytes was significantly associated with chlamydial infection ($\chi^2 = 19.52$, degrees of freedom = 2, $p = 0.001$). On the basis of this combination of cytologic changes, 129 of 148 (87%) cases were correctly classified as positive or negative for chlamydia as outlined in Table II. However the sensitivity

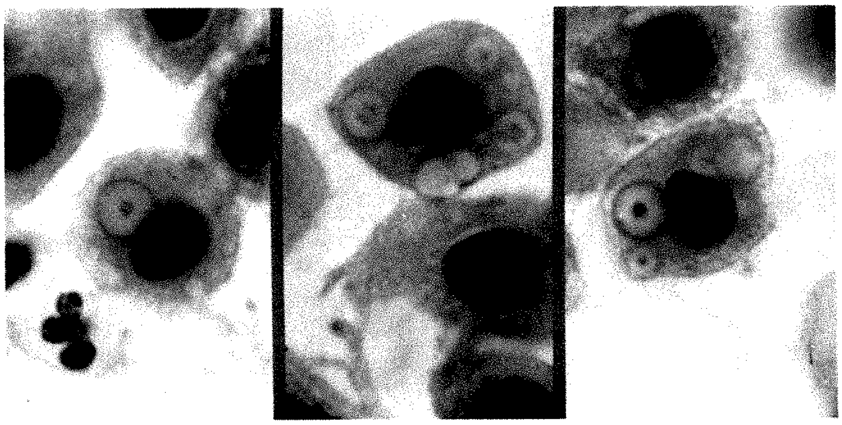

Fig. 6. Large densities or "inclusions" in metaplastic cells from a *C. trachomatis*–negative patient. (×400.)

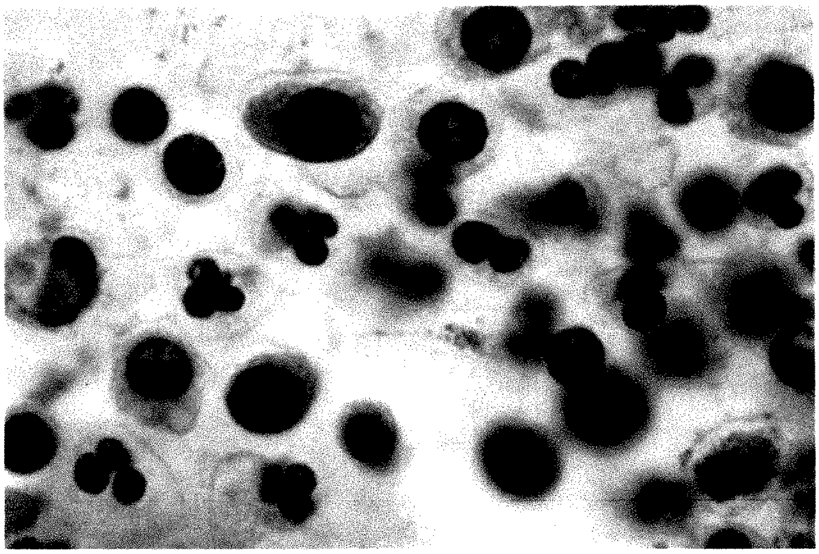

Fig. 7. Lymphocytic infiltration in a smear from a *C. trachomatis*–positive patient. (×400.)

of this combination of cytologic findings for predicting *C. trachomatis* infection was found to be low (17.4%). When cases without endocervical cells (nonrepresentative) were removed for this analysis, the sensitivity of these same cytologic criteria in the prediction of chlamydial isolation results was again found to be low.

Comment

We found that cytologic testing was not useful in making a definitive diagnosis of endocervical chlamydial infection. "Inclusions" that have been reported by others[5, 14] in association with cytoplasmic vacuoles were not related to chlamydial isolation in our study. The lack of sensitivity for so-called "inclusions" may be due to differences in our population, to differences in our criteria for diagnosis of chlamydial infection, or to the fact that "inclusions" are not consistently demonstrated with the techniques of fixation and the staining methods used for the Papanicolaou smear.

The population we studied was unusual in that it was limited to sexually active adolescents attending our Teen Clinic. There was no preselection on the basis of clinical evidence of cervicitis. It is possible that the developing transformation zone in these young women responds in a different way to chlamydial infection. Another consideration is that a first infection or the early cytologic manifestations of infections are different from reinfection or late cytologic manifestations.

Chlamydial isolation was used to determine the presence of disease in our patients, whereas earlier reports of "inclusions" diagnostic for chlamydia on Papanicolaou smear described other criteria.[5, 14] These criteria were based on a variety of cytologic staining and immunofluorescent techniques and did not always include

Table I. Cytologic findings compared with chlamydial isolates

	Total (N = 148)		*Chlamydia trachomatis positive (N = 23)*		*Chlamydia trachomatis negative (N = 125)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*p**
Cytologic inflammation	65	44	17	74	48	38	0.003†
Lymphocytes	3	2	3	13	0	0	0.001†
Nuclear changes in metaplastic cells	15	10	6	26	9	7	0.02†‡
Vacuoles in metaplastic cells	18	12	4	17	14	11	NS
Inclusions	13	9	3	13	10	8	NS
Plasma cells	2	1	2	7	0	0	NS

*χ^3 Analysis.

†Significant association after controlling separately for *T. vaginalis, N. gonorrhoeae,* yeast, and viral inclusions.

‡Nonsignificant in logistic regression analysis; see text under Results.

Table II. Prediction of *Chlamydia trachomatis* cervical infection on basis of joint cytologic findings of inflammation and lymphocytes

Endocervical isolation	*Predicted infection*	
	Negative	*Positive*
Negative	125	0
Positive	19	4

Sensitivity = 17.4%. Specificity = 100%. Correct classification of *Chlamydia trachomatis*–positive and –negative cases = 87%.

chlamydial isolation as a diagnostic criterion. These differences in methodology have been associated with decreased sensitivity and specificity when compared to those with chlamydial isolation.[4] Other authors*, [15] have reported no significant association between the Papanicolaou smear findings of "inclusions" and chlamydial isolation; this conclusion is similar to our findings. Sample variability among the specimens collected for chlamydial isolation and those collected for cytologic testing may partly explain the discrepancies. Separate but consecutive samples were obtained at the same clinic visit for *C. trachomatis* isolation, other microbiologic studies, and cytologic testing. Considerable discrepancies in cytologic findings of dysplasia and carcinoma in situ have been reported in a comparison of consecutive samples collected at the same time in the manner described by us.[16] It is a reasonable assumption that similar discrepancies could occur in *C. trachomatis* isolations on sequential samples.

The "inclusions" described and illustrated by Gupta et al.[5] in Papanicolaou-stained smears differ somewhat from the morphologic features of the organism in smears and cell cultures stained with Giemsa. Cytoplasmic vacuoles associated with mucin production or vacuoles caused by inflammation and degeneration with particulate matter overlying the vacuole may resemble chlamydial inclusions.

Dorman et al.[15] noted that sensitivity of the Papanicolaou smear for chlamydia-associated changes was reduced in unsatisfactory smears that did not contain endocervical columnar cells or metaplastic cells, a finding that is consistent with ours. In smears having no endocervical columnar cells, the significant cytologic features either were absent or occurred much less frequently. Smears lacking endocervical columnar cells are unsatisfactory for either routine examination or evaluation of the cytologic features we have described in chlamydial infection.[4] This does not help to explain our failure to find "inclusions" significantly related to chlamydial isolation since all but one of 23 women classified as chlamydia positive had endocervical columnar cells in smears.

The cellular findings that had a significant relationship to chlamydial isolation in our study included cytologic inflammation, lymphocytes, and nuclear changes in metaplastic cells. Studies with chlamydial isolation used as the criterion for presence of disease have reported similar findings.[7, 13-15, 17] However, when we used stepwise logistic regression analysis to determine the joint association of these three cytologic findings with *C. trachomatis* infection, nuclear changes in metaplastic cells was no longer found to be significantly related. While such findings do not support the promotion of the Papanicolaou smear as a definitive diagnostic tool for chlamydial infection, it may be possible to use the cytologic pattern we have identified to define a population with a high prevalence of chlamydial infection. Verification with culture and treatment for chlamydial infection may then be justified in such a population.

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*Kiviat N, Paavonen J, Brockway J, et al. Diagnosis of *C. trachomatis* using routine Papanicolaou cervical smears. Paper presented to the International Society for Sexually Transmitted Disease Research, Seattle, Washington, August 1-3, 1983.

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Cefotaxime treatment for women with community-acquired pelvic abscesses

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Forty-one women with pelvic abscesses complicating salpingitis were treated with parenteral cefotaxime, a newer cephalosporin. Abscesses ranged in size from 4 by 4 to 13 by 15 cm, and in 10 women (24%) they were ≥10 cm. *Neisseria gonorrhoeae* was recovered from the endocervix in 17 women (41%). A mean of 26.7 gm of cefotaxime was given over a mean of 6.5 days, and operation was not required during initial therapy. Only two women (5%) required the addition of another antimicrobial. Chronic pelvic pain and recurrent infection were infrequent during the 31- to 43-month follow-up period. Five women (12%) were readmitted for elective surgical therapy because of persistent or recurrent adnexal mass 1 to 33 months following study entry. Six (15%) women became pregnant and were delivered of their infants a mean of 25 months following cefotaxime therapy. (Am J Obstet Gynecol 1985;151:771-7.)

Key words: Salpingitis, pelvic abscess, cefotaxime

The most significant community-acquired infection treated by the obstetrician/gynecologist is acute salpingitis complicated by pelvic abscess. Successful medical therapy is reportedly followed by a significant infertility rate, chronic pain, and a high incidence of subsequent pelvic operations. Rupture of this inflammatory process is life-threatening and surgical therapy for ruptured or unruptured abscesses almost invariably includes re-

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moval of uterus, tubes, and ovaries. Prospective data relating to therapy of and consequences of pelvic abscesses are rare, and none were available from our hospital.

The development of newer antimicrobials and their application in the treatment of this disease may alter not only the success rate of medical management but also the clinical course following therapy. Cefotaxime is a β-lactamase stable[1] semisynthetic cephalosporin with an expanded spectrum of bactericidal activity including most anaerobes.[2-4] It has proved clinical efficacy (96.4%) comparable to that of clindamycin and gentamicin in the treatment of women with post–cesarean section endomyometritis.[5] A prospective study was designed to: (1) evaluate the efficacy of cefotaxime in the treatment of women sequentially admitted for therapy of pelvic abscess complicating acute salpingitis and (2) determine the long-term consequences or natural history of this disease. Results with this newer cephalosporin are encouraging.

Material and methods

Women admitted to this study complained of lower abdominal and pelvic pain and had an oral temperature of at least 38° C (100.4° F). They had abdominal tenderness, pain with cervical motion, and uterine and adnexal tenderness associated with the presence of a palpable, tender, fluctuant adnexal or pelvic mass considered to be a pyosalpinx, tuboovarian abscess, or pelvic abscess. None of the women had an abscess that dissected the upper one third of the rectovaginal septum; our policy is to drain such abscesses. All gave informed, written consent. Reasons for exclusion were: (1) history of allergy to cephalosporins or immediate hypersensitivity reaction to penicillin, (2) receipt of any investigational drug or probenecid during the preceding 2 weeks, (3) receipt of antimicrobials within 3 days prior to therapy, and (4) significant renal or hepatic disease. Laboratory tests, performed prior to therapy, on the third day of therapy, and following therapy included complete blood count, prothrombin time, partial thromboplastin generation time, direct and indirect Coombs tests, measurement of serum creatinine, total protein, albumin, uric acid, inorganic phosphorus, calcium, glucose, alkaline phosphatase, total bilirubin, glutamic oxaloacetic transaminase, and urea nitrogen, and complete urinalysis. Blood and urine were collected for culture. A swab culture for *Neisseria gonorrhoeae* was taken from the endocervix of each subject prior to therapy. Peritoneal fluid was collected by culdocentesis from eight women (19.5%) with lateral masses and was transported in Anaport vials (Scott Laboratories, Fiskeville, Rhode Island). We do not routinely perform culdocentesis in women with a pelvic abscess(es); if there was a lateral mass and a question about the cause of peritoneal irritation, culdocentesis was performed. Culdocentesis specimens were processed as previously described.[6]

Cefotaxime was given intravenously in 2 gm doses every 8 hours and the women were seen at least twice daily by the physician-investigator. Twenty-nine women (71%) had pelvic and abdominal ultrasonography performed and interpreted by one of us (R. S.-R.) using a static B scanner (Rohnar 7000, Santa Ana, California). The other 12 had no sonography because of mechanical problems. Clinical response was defined as progressive diminution in symptoms, abdominal and pelvic tenderness, mass size, and return of oral temperature to ≤37.5° C (99.5° F). Clinical failure was defined as persistence of abdominal and pelvic pain and tenderness with fever after 72 hours of therapy or signs of worsening infection including increasing pain, mass size, tenderness, and temperature at any time. When this occurred, chloramphenicol, 50 mg/kg/day, was added intravenously in divided doses. Women were discharged after their temperature was <37.5° C for 24 to 48 hours and were instructed to take tetracycline or cefalexin, 500 mg orally four times daily. They were seen in the clinic 3 to 14 days after hospital discharge. A 30-month minimum period was selected for follow-up to assess the clinical outcome following treatment for this infection.

Continuous data were analyzed by Student's t, paired Student's t, or Mann-Whitney test. Discrete data were compared by χ^2, Welch's approximation, and Kruskal-Wallis tests.

Results

Between January, 1980, and January, 1981, 41 women were entered into this study. Ages ranged from 15 to 36 years and 23 (56%) were black, 16 (39%) were Caucasian, and one each (2.5%) was American Indian and Mexican-American. Mean parity was 1.1, seven (17%) were nulligravid, and 11 (27%) were practicing contraception. Only four were using an intrauterine contraceptive device and three of these women had bilateral abscesses. Twenty (49%) gave a history of previous treatment for either acute pelvic infection or asymptomatic gonococcal colonization. These were almost evenly divided. The mean duration of symptoms before presentation to our emergency room was 6.5 days and the mean interval since the last menstrual period was 16.5 days. Nine women (22%) had bilateral lower-quadrant abdominal peritonitis and 13 others (32%) had four-quadrant peritonitis; all women had pelvic peritonitis. Twenty-five women (61%) had bilateral abscesses while eight (19.5%) had central and eight had unilateral abscesses. Clinical variables were similar for women irrespective of presence of bilateral, unilateral, or central abscesses, although central abscesses

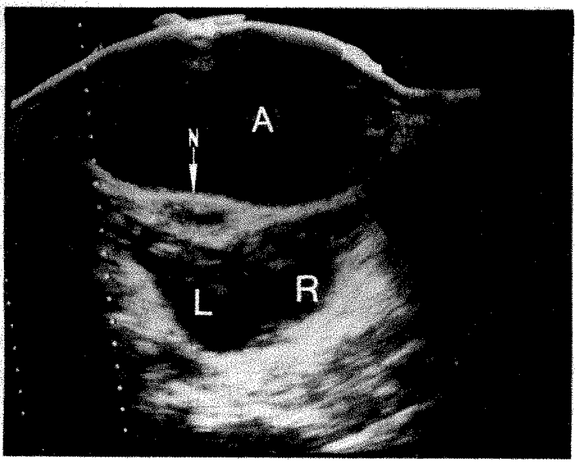

Fig. 1. Typical sonogram of bilateral tuboovarian abscess. *A* = Sonolucent urine-filled bladder; *N-arrow* is pointing to the uterus, which is between the bladder and a left tuboovarian abscess *(L)*; *R* designates a right tuboovarian abscess. Sonographically, abscesses are usually sonolucent structures with indistinct borders and internal echoes representing debris or septa.

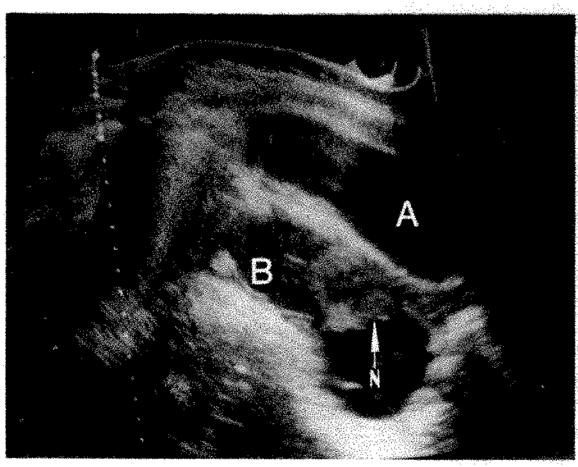

Fig. 2. Sagittal sonogram of pelvis and lower abdomen showing the bladder *(A)* and abdominal extension of 13 by 15 cm pelvic/abdominal abscess *(B)*. *N-arrow* is the posterior pelvic portion of the abscess with *arrow* pointing at the uterus, which lies between it and the bladder.

were larger than unilateral abscesses. There was close agreement between clinical estimation of abscess size and sonographic measurement unless the inflammatory mass was more than 12 weeks' gestational size. Abscess size was sonographically smaller in these instances. Typical sonograms appear in Figs. 1 and 2.

Most women had daily maximal temperature readings that were similar to those observed prior to admission for at least the first 72 hours of therapy, after which time the temperature decreased to below 37.5% C and remained there. Two women (5%) experienced failure of the original therapy and required the addition of chloramphenicol. Both had an inflammatory mass the size of a 14-week gestation with bilateral abscesses. Sonographic measurements of the abscesses were 5 by 4 by 3 cm each in one patient and 9 by 8 by 6 and 6 by 6 cm in the other. Various clinical, laboratory, and treatment variables were analyzed by abscess size and are presented in Tables I and II. Variables in Table I were statistically similar ($p > 0.05$, Kruskal-Wallis or χ^2) with no evidence of trend by abscess size. Those presented in Table II, however, do indicate an influence of mass size on the clinical course. Patient numbers in each group were small enough in the first three categories that statistical significance did not exist, but trends were present. Therapy duration for women with a mass ≥ 10 cm was significantly longer than that for the women in other groups ($p = 0.005$, Kruskal-Wallis). Total drug dose for those women was also significantly greater than that for women with an abscess ≤ 6 cm ($p = 0.001$, Kruskal-Wallis) but not for those whose abscess measured 7 to 9 cm. The incidence of the presence of a mass at hospital discharge was significantly and progressively greater with increasing abscess size when the first two categories are combined ($p < 0.001$, χ^2). Interval to absence of a palpable mass at a subsequent clinic visit was also related to abscess size ($p < 0.001$), Kruskal-Wallis). All masses that were ≤ 6 cm at admission were not palpable at examination 2 weeks after discharge (Table II).

Thirteen isolates were identified from five of the eight peritoneal fluid specimens; eight were aerobic and five anaerobic, including *Bacteroides fragilis*. The minimal inhibitory concentration for cefotaxime to 90% of the isolates, including *Bacteroides fragilis*, was 2 μg/ml. The highest was 4 μg/ml and was seen with *Enterobacter aerogenes*. The organisms contained in the abscesses of the 41 women were essentially unknown.

Mean hemoglobin concentration (± SD) at admission was 12.7 ± 1.8 gm/dl and the mean leukocyte count was $14.2 \pm 4.5 \times 10^3/\text{mm}^3$. At discharge, these values were 11.7 ± 1.5 and 8.6 ± 3.0, respectively. Prothrombin time and partial thromboplastin generation time means decreased during therapy and platelet count means increased; bleeding was not observed. In four instances (10%), alkaline phosphatase values were elevated at study entry and returned to normal during therapy. In four others, transitory elevation during therapy was observed. Serum transaminase was elevated at entry for two women and became normal during therapy; for five others, there was transitory elevation during therapy. Coombs tests did not become positive and other parameters measured were generally within normal limits. Eight women (19.5%) developed mild to moderate phlebitis that required changing the site of intravenous administration and possibly local therapy. One woman complained of burning eyes and pruritus; therapy was continued, and the symptoms

Table I. Statistically similar* variables and means by pelvic abscess size (incidence or mean ± SD)

	Abscess size			
Variable	*<6 cm (n = 6)*	*6 cm (n = 8)*	*7-9 cm (n = 17)*	*≥10 cm (n = 10)*
Incidence (%)	15	19.5	41.5	24†
Age (yr)	23.2 ± 7.3	20.9 ± 2.5	22.9 ± 4.3	21.6 ± 5.4‡
Days of symptoms	8.8 ± 9.4	4.2 ± 6.8	5.8 ± 5.3	8.4 ± 6.4‡
Previous pelvic infection or gonococcal colonization (%)	33	37.5	59	50†
Maximal temperature (° C)	38.8 ± 0.4	38.8 ± 0.5	38.9 ± 0.5	38.9 ± 1.1‡
Abdominal peritonitis (%)	50	50	59	50†

*$p > 0.05$.

†χ^2.

‡Kruskal-Wallis.

Table II. Variables relating clinical course to pelvic abscess by size (incidence or mean ± SD)

	Abscess size				
Variable	*<6 cm (n = 6)*	*6 cm (n = 8)*	*7-9 cm (n = 17)*	*≥10 cm (n = 10)*	*Significance (p)*
Positive *N. gonorrhoeae* culture (%)	67	50	35	30	NS*†
Days to last menstrual period	11.2 ± 3.3	13.8 ± 5.7	15.6 ± 9.8	18.9 ± 11.5	NS‡
Leukocyte count (× $10^3/mm^3$)	13.2 ± 4.6	13.8 ± 3.7	14.4 ± 5.2	15.0 ± 4.5	NS‡
Days of therapy	5.3 ± 0.7	5.1 ± 0.9	6.4 ± 1.5	8.7 ± 3.1	0.005§
Total drug dose	17.4 ± 4.9	19.2 ± 5.0	26.9 ± 8.2	37.0 ± 16.4	0.001§
Palpable mass at hospital discharge (%)	17	12.5	59	100	<0.001†
Interval to no masses (wk)	1.4 ± 0.5	1.4 ± 0.9	3.9 ± 3.5	10.0 ± 4.3	<0.001§

*NS = Not significant ($p > 0.05$).

†χ^2.

‡Welch's approximation.

§Kruskal-Wallis.

disappeared. Cefotaxime was discontinued in one woman after 7 days because of presumed "drug fever." Eosinophilia and temperature returned to normal.

Thirty-nine women (95%) were seen in the clinic during the 43-month interval following study entry. Only two women (5%) had another documented episode of acute pelvic infection following hospital discharge and these occurred 4 and 33 months later. Because of the time intervals, they most likely represented reinfection rather than recurrence. Masses were not palpated at an interval clinic visit for either patient but were present at readmission. Five women (12%) with no desire for future fertility had uterus, tubes, and ovaries removed because of persistent adnexal mass 1 to 33 months following treatment. None had experienced failure of original treatment and three had undergone previous tubal ligation. All specimens had histologic evidence of chronic tuboovarian inflammation.

Variables from these five women were compared to those from six women (15%) who became pregnant during the 31- to 43-month interval following study entry and are presented in Table III. Five of the six women who became pregnant were delivered of healthy infants at term and one underwent elective termination. Four of these women had been treated for bilateral abscesses and one had a central abscess. The sixth woman had a unilateral abscess at study entry and underwent an interval unilateral salpingo-oophorectomy because of an asymptomatic adnexal mass (hydrosalpinx) 22 months following therapy. Chronic inflammation of that tube and ovary were present at histologic examination: the other tube and ovary appeared normal, and she had never used an intrauterine contraceptive device. Five of the six women underwent sonography and had sonographic findings compatible with pelvic abscess. Four of the six women had a palpable pelvic mass at discharge from the hospital following initial therapy. The mean interval between treatment and delivery or termination was 25 months. Eight women were maintained on a regimen of oral contraceptives after discharge from the hospital. When these eight women are combined with the three who had undergone previous tubal ligation, a pregnancy rate of 20% (six of 30) was observed.

Table III. Variables in women with pelvic abscess who ultimately underwent hysterectomy or became pregnant (mean ± SD)

Variable	*Hysterectomy* *(n = 5)*	*Pregnancy* *(n = 6)*	*Significance* *(p)*
Age (yr)	28.0 ± 5.5	20.5 ± 2.3	0.026*
Days of cefotaxime therapy	6.3 ± 1.9	6.5 ± 2.0	NS†‡
Total cefotaxime dose (gm)	25.0 ± 9.4	27.0 ± 8.0	NS‡
Original abscess size			
≤6 cm	1	0	NS*
7-9 cm	3	3	NS*
≥10 cm	1	3	NS*

*Welch's approximation.
†NS = Not significant ($p > 0.05$).
‡Student's *t*.

Comment

The pathogenesis and microbiologic features of acute pelvic inflammatory disease have been documented.[7-11] Pathogens recovered from women with abscesses confirm the polymicrobial nature of this infection as well.[11-19] Reported accuracy of clinical diagnosis of acute salpingitis in the absence of masses ranges from about 65% to 90%[8, 20, 21] according to studies in which laparoscopy was used for confirmation. Correlation between ultrasonographic diagnosis of pelvic abscess and laparoscopic or laparotomy findings ranges from 76% to 100%.[22-27] The obvious problem with studies such as the current one is that there is no surgical confirmation of the diagnosis. Accuracy of ultrasonographic diagnosis approaching 100%,[26, 27] coupled with clinical requirements for study admission and histologic confirmation in women who underwent later operation, satisfied us of the clinical accuracy of the diagnosis.

As stated by Collins and Jansen*: "Before the era of sulfa drugs and penicillin, when our therapeutic medical regimen was limited to antipyretics, sedatives, bed rest, heat applied to the pelvis, and general supportive measures, a large number of cases of severe pelvic infection underwent resolution. Even in some cases of tuboovarian abscess, the abscess became sterile and a hydrosalpinx alone, or involving not only the tube, but the tube and part or all of the ovary, resulted. Therefore, one cannot say that, because an inflammatory mass in the pelvis cools or even partially regresses under antibiotic therapy, an abscess was not present." Kaplan et al.[28] reported that a retrospective study conducted in their hospital indicated a high incidence of chronic pelvic pain, recurrent pelvic infections, and almost complete infertility in women who were treated medically for pelvic abscess. For that reason, in a rare prospective study of therapy for women with a pelvic abscess, they electively treated 71 women with operation after preparation with fluid and antibiotics. They stated that operation consisted of a total abdominal hysterectomy and bilateral salpingo-oophorectomy. Chronic pain and recurrent infections may have been prevented, but 40% of the 71 women required blood, 17% sustained major operative injuries, and 32% developed other complications such as wound infection, cuff abscess, and pyelitis. Other authors[13, 26, 29-31] confirmed a significant requirement for hysterectomy in their retrospective studies. In two other retrospective studies,[19, 32] colpotomy was included as a part of early therapy; in spite of this, 33% to 50% of women ultimately required hysterectomy and castration for definitive therapy. The mean hospital stay for women in these latter two studies was 13.4 days, so colpotomy did not appear to guarantee a short hospital stay. Ginsburg et al.[30] reported that pelvic infection recurred in 80% of women treated medically for tuboovarian abscess and that 86% of the women treated medically had chronic pain. Franklin et al.[33] found the conservative approach to this infection "successful." They performed colpotomy when the upper one third of the rectovaginal system was involved by a dissecting abscess (35 of 120 women). Twelve women (10%), however, required definitive surgical therapy initially and 19 (16%) required subsequent definitive operation for persistent mass, pain, or menometrorrhagia. Data reported by Landers and Sweet[26] a decade later indicated that 25% of women required operation as a part of original therapy. An additional 31% of those followed up after original therapy required a subsequent laparotomy. Conception rates of about 10% were reported by most authors when potential for fertility was retained.

Prior to this prospective study, women admitted to our hospital for therapy of pelvic abscess were empirically treated with a combination of antimicrobials. Initially, these included penicillin and tetracycline, then penicillin or cephalosporin and aminoglycoside, as trends in therapy changed. Clindamycin or chloramphenicol was required in addition in 16% to 50% of cases when initial combination therapy failed and was added to initial therapy if warranted by the woman's clinical condition. Ten of 86 women (11.6%) hospitalized and treated during an 18-month period in 1976 and 1977 required hysterectomy and bilateral salpingo-oophorectomy as a part of their initial treatment because of failed antimicrobial therapy. When medical therapy was successful, removal of uterus, tubes, and ovaries was frequently performed electively 6 weeks later if fertility was not desired because of the "natural history" of the infection.

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*Collins CC, Jansen FW: Treatment of pelvic abscesses. Clin Obstet Gynecol 1959;2:512.

Historically the infection described is one of considerable significance and consequence. No explanation is apparent for variations in persistence of pain, recurrence of acute infections, or the varied requirement for surgical therapy reported by some investigators. Chronic pain and recurrent infection occurred in only 10% of women in this prospective study, however, and more women conceived than subsequently required hysterectomy. Cefotaxime therapy was associated with excellent initial success in the medical management of this disease. Failures that occurred were in women with masses that were 14 weeks' gestational size. It is our current practice to treat women with abscesses larger than 12 weeks' gestational size with combination antimicrobials including an anaerobic-specific agent.

It is unlikely that oral tetracycline (or cephalexin) can be given significant credit, if any, for the long-term results of therapy. Women hospitalized and treated for acute pelvic inflammatory disease in our hospital, including those with an abscess, are now discharged on a regimen of oral tetracycline because of the possible contribution of chlamydia to this disease. However, Sweet (personal communication) has been unable to isolate chlamydia from abscesses. Our long-term clinical management of women treated for this infection has been modified by this prospective study since chronic pain, persistent mass, and recurrent infections were infrequent. Routine elective operation 6 weeks following therapy has been abandoned.

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Factors affecting the sex differential in neonatal mortality: The role of respiratory distress syndrome

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We investigated factors affecting the sex differential in neonatal mortality rate using linked birth and death certificates of more than 300,000 infants born in Georgia between 1974 and 1977. The neonatal mortality rate was higher in male infants than in female infants (relative risk = 1.23, $p < 10^{-8}$) and was most pronounced for infants weighing between 1501 and 2500 gm (relative risk = 1.78, $p < 10^{-8}$). This differential persisted after adjustment was made for effects of several prenatal and labor-related factors. The male excess in neonatal mortality rate was most prominent during the first week of life and was found for several perinatal disorders. Respiratory distress syndrome–related mortality showed the largest male excess (relative risk = 1.57, $p < 10^{-8}$) and was most pronounced in infants weighing between 1501 and 2500 gm (relative risk = 2.78, $p < 10^{-8}$), in whom it accounted for roughly 60% of the excess. Since previous studies have shown that male infants have a higher incidence of respiratory distress syndrome but not a higher case-fatality rate, we suggest that slower lung maturation among male fetuses is a major contributing factor to the sex differential in neonatal mortality. (AM J OBSTET GYNECOL 1985;151:777-82.)

Key words: Infant mortality, neonatal mortality, respiratory distress syndrome, sex differences

The neonatal mortality rate of male infants is known to be higher than that of female infants. This excess has been demonstrated for several specific neonatal conditions, suggesting general biologic weakness of the male infant after birth.[1,2] Respiratory distress syndrome (RDS)–hyaline membrane disease (HMD) has been shown to have a higher male : female death ratio than any other major cause of infant death.[3] Other demographic and clinical factors known to be powerful predictors of neonatal mortality include birth weight,[4] maternal age, and complications of pregnancy and labor.[5]

In this study, we used linked birth and death certificates from a total population to investigate whether sex-specific differences in prevalence of these other risk factors could contribute to the sex differential in neonatal mortality. In addition, we estimated the relative proportion of the various causes of death, including RDS/HMD, in the male excess.

Methods

The Vital Records Division, State of Georgia, has linked infant birth and death certificates in an effort to establish maternal and infant risk factors for infant mortality.[5] A search for unregistered neonatal deaths was undertaken by using hospital records, and those found were then linked to birth certificates.[6] A neonatal death was defined as a death of a live-born infant occurring at less than 28 days of age.

In this study, we examined the cohort of 317,404 live infants born in Georgia between 1974 and 1977. This study was limited to these years to assure consistency in the cause of death codes because of the changes in the International Classification of Diseases, adapted (ICDA) codes that occurred in 1978.[7] Male and female live-born infants were compared for maternal demo-

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Table I. Sex differences in neonatal mortality rates by birth weight distribution, Georgia, 1974 to 1977

Birth-relative weight (gm)	*Male infants*			*Female infants*			Risk (male/ female)
	Live births		*Neonatal mortality rate**	*Live births*		*Neonatal mortality rate**	
	No.	*%*		*No.*	*%*		
≤750	461	0.3	974.0	483	0.3	968.9	1.01
751-1000	468	0.3	747.9	425	0.3	632.9	1.18†
1001-1500	1,194	0.7	350.1	1,188	0.8	247.5	1.42†
1501-2000	2,696	1.7	98.3	2,853	1.9	54.7	1.80†
2001-2500	8,064	5.0	23.7	9,632	6.2	13.4	1.77†
≥2501	146,360	89.8	3.2	136,385	88.3	2.6	1.22†
Unknown	3,762	2.3	43.3	3,433	2.2	29.4	1.47‡
Total							
Observed	163,005	100.0	14.1	154,399	100.0	11.5	1.23§
Adjusted			14.5			10.6	1.37‖

*Deaths per 1000 live births.

†$p < 0.001$.

‡$p < 0.01$.

§$\chi^2 = 43.4$, $p < 10^{-8}$, 95% confidence limits 1.16 to 1.31.

‖Adjusted for birth weight differences by direct standardization with all the population as standard; Mantel-Haenszel summary $\chi^2 = 107.0$, $p < 10^{-8}$.

Table II. Sex differences in neonatal mortality rates by prenatal risk score, Georgia, 1974 to 1977

Risk score*	*Male infants*			*Female infants*			Relative risk (male/female)
	Live births		*Neonatal mortality rate†*	*Live births*		*Neonatal mortality rate†*	
	No.	*%*		*No.*	*%*		
None	89,091	54.7	8.1	84,437	54.7	6.2	1.32‡
1-10	57,915	35.5	13.7	54,809	35.5	11.2	1.22‡
11-20	6,539	4.0	34.1	6,108	4.0	26.0	1.31§
21-30	3,479	2.1	42.8	3,188	2.1	35.5	1.21
31-40	4,717	2.9	59.2	4,608	3.0	48.6	1.22‖
≥41	1,264	0.8	103.6	1,249	0.8	112.1	0.92
Total	163,005	100.0	14.1	154,399	100.0	11.5	1.23¶

*Risk score based on a weighted combination of the following factors: maternal age (<20 or >35 yrs), black race, multiple gestations, history of prematurity, history of fetal loss, complications related to pregnancy, and complications not related to pregnancy.

†Deaths per 1000 live births.

‡$p < 0.0001$.

§$p < 0.01$.

‖$p < 0.05$.

¶$\chi^2 = 43.4$, $p < 10^{-8}$, 95% confidence limits = 1.16 to 1.31.

graphic and obstetric factors. In addition, sex-specific neonatal mortality rates were compared within each birth weight category and relative risk of neonatal death was computed from the ratio of the male neonatal mortality rate divided by the female neonatal mortality rate. Crude relative risks were adjusted by using direct standardization for the presence of other maternal factors and a summary χ^2 was calculated across levels of a factor using the Mantel-Haenszel technique. Finally, we compared sex-specific neonatal mortality rates within categories of increasing risk of neonatal death on the basis of prenatal factors previously found to independently predict neonatal mortality in the same population with a discriminant function analysis used to weight the various factors.[5] These factors were: (1) maternal age (<20 and >35 years), (2) race (black), (3) past history of prematurity, (4) past history of fetal loss, (5) multiple gestations, (6) complications related to pregnancy (toxemia, Rh sensitization, syphilis, rubella, other viral infections, anemia, and other), and (7) complications not related to pregnancy (diabetes, symptomatic heart disease, chronic renal disease, hypertension, injury, tumor or operation during pregnancy, and other).

Sex-specific neonatal mortality rates were also compared for age at death and for various causes of mortality coded according to the eighth revision of the

Table III. Sex differences in neonatal mortality rates by presence of delivery complications, Georgia, 1974 to 1977

Delivery complication	Male infants: Live births No.	Male infants: Live births %	Male infants: Neonatal mortality rate[*]	Female infants: Live births No.	Female infants: Live births %	Female infants: Neonatal mortality rate[*]	Relative risk (male/female)	Adjusted[†] relative risk (male/female)
Labor complications[‡]								
Yes	35,099	21.5	35.4	31,951	20.7	32.5	1.09[§]	1.33[‖]
No	127,906	78.5	8.3	122,448	79.3	6.0	1.38[‖]	
Emergency cesarean section								
Yes	8,861	5.4	21.0	7,241	4.7	18.4	1.14	1.23[‖]
No	154,144	94.6	13.7	147,158	95.3	11.1	1.23[‖]	
Birth trauma[¶]								
Yes	357	0.22	81.2	271	0.18	66.4	1.22	1.23[‖]
No	162,648	99.78	14.0	154,128	99.82	11.4	1.23[‖]	

[*] Deaths per 1000 live births.

[†] Adjusted for each factor by direct standardization with the whole population used as standard.

[‡] Labor complications included abruptio placentae, placenta previa, cord collapse or compression, dystocia, breech delivery, maternal hypotension, prolonged labor, intrapartum fever, and other.

[§] $p < 0.05$.

[‖] $p < 10^{-8}$.

[¶] Birth trauma included central nervous system or other injury during labor.

Table IV. Sex differences in neonatal mortality rates by age at death, Georgia, 1974 to 1977

Age at death	Male infants (n = 163,005): No. of deaths	Male infants (n = 163,005): Neonatal mortality rate[*]	Female infants (n = 154,399): No. of deaths	Female infants (n = 154,399): Neonatal mortality rate[*]	Relative risk (male/female)
≤6 hr	921	5.65	717	4.64	1.22[†]
7-23 hr	275	1.69	219	1.42	1.19[‡]
1-6 days	760	4.66	535	3.47	1.35[†]
7-28 days	284	1.74	252	1.63	1.07
Unknown (neonatal)	57	0.35	50	0.32	1.08

[*] Deaths per 1000 live births.

[†] $p < 0.0001$.

[‡] $p < 0.02$.

ICDA.[7] Although individual ICDA codes were examined in the analysis, these were grouped into several categories of causes for data presentation because of similar trends in sex-specific neonatal mortality rates (for instance, infectious diseases) and because of small numbers of deaths in some categories (for instance, other). The contribution of each cause of death to the overall excess in male neonatal mortality rates was calculated by dividing the male excess in the neonatal mortality rates from each specific cause by the male excess in neonatal mortality rates for all causes according to the following formula:

Percent contribution of each cause to overall male excess in NMR =

$$\frac{(\text{male CSNMR} - \text{female CSNMR})}{(\text{total male NMR} - \text{total female NMR})} \times 100\%$$

where NMR is neonatal mortality rate and CSNMR is cause-specific neonatal mortality rate.

Results

Effect of birth weight. The overall male neonatal mortality rate (14.1 per 1000 live births) was significantly greater than the female neonatal mortality rate (11.5 per 1000 live births, relative risk = 1.23, $p < 10^{-8}$) (Table I). Moreover, although male infants tended to weigh more than female infants, the male excess in neonatal mortality rates was consistent within each birth weight category except in that for infants weighing ≤750 gm. The excess was most pronounced for infants weighing between 1501 and 2500 gm (relative risk = 1.78, $p < 10^{-8}$). Because male sex was associated with both increased birth weight and increased

Table V. Sex differences in neonatal mortality rates by cause of death, Georgia, 1974 to 1977

		Male infants (n = 163,005)		*Female infants (n = 154,399)*			
Cause of death	*ICDA code*	*No. of deaths*	*Neonatal mortality rate*	*No. of deaths*	*Neonatal mortality rate*	*Relative risk (male/female)*	*95% Confidence limit*
RDS-HMD	776.1, 776.2	511	3.13	308	1.99	1.57*	1.36-1.81
Birth injuries	764-768, 772	124	0.76	86	0.56	1.37†	1.04-1.80
Infections	00-136, 320, 470-474, 480-486	166	1.02	119	0.77	1.32†	1.04-1.67
Prematurity, unqualified	777	210	1.29	160	1.04	1.24†	1.01-1.53
Maternal complications (pregnancy and childbirth)	630-669, 760-763, 769	215	1.32	180	1.17	1.13	0.93-1.38
Congenital anomalies	74-75	277	1.70	239	1.55	1.10	0.92-1.31
Neonatal asphyxia	776.0, 776.3-776.9	287	1.76	253	1.64	1.07	0.91-1.27
Unknown	?	251	1.54	232	1.50	1.03	0.86-1.23
Others	Rest.	260	1.60	196	1.27	1.26†	1.04-1.51
Total		2,301	14.1	1,773	11.5	1.23[1]	1.16-1.31

*$p < 10^{-8}$.
†$p < 0.05$.

Table VI. Sex differences in neonatal mortality rates related to RDS, by birth weight distribution, Georgia, 1974 to 1977

	Male infants		*Female infants*			
Birth weight (gm)	*No. of deaths*	*Neonatal mortality rate**	*No. of deaths*	*Neonatal mortality rate**	*Relative risk (male/female)*	*Percent excess neonatal mortality rate due to RDS*
≤750	32	69.4	44	91.1	0.76	–
751-1000	82	175.2	69	162.4	1.08	11.1
1001-1500	130	108.9	80	67.3	1.62†	40.5
1501-2000	122	45.3	55	19.3	2.35†	59.6
2001-2500	67	8.3	24	2.5	3.33†	56.3
≥2501	48	0.33	23	0.17	1.94‡	28.1
Unknown	30	7.8	13	3.8	2.11§	28.8
Total						
Observed	511	3.13	308	1.99	1.57‖	43.2
Adjusted		3.24		1.95	1.67¶	

*Deaths due to RDS per 1000 live births in each weight category.
†$p < 0.0001$.
‡$p < 0.01$.
§$p < 0.05$.
‖$\chi^2 = 40.0$, $p < 10^{-8}$.
¶Direct standardization with the whole population used as standard; Mantel-Haenszel summary $\chi^2 = 55.0$, $p < 10^{-8}$.

mortality rate, adjustment for birth weight increased the estimated risk in male infants (adjusted relative risk = 1.37, $p < 10^{-8}$).

Effect of prenatal risk factors. Male and female infants had an almost identical distribution of prenatal risk levels (Table II). For both male and female infants, the neonatal mortality rate increased sharply with the increasing risk score. However, the male excess in neonatal mortality rates persisted in all categories of a priori risk except those in which the mothers were at highest risk.

Effect of delivery complications. The births of male infants involved slightly more delivery complications than those of female infants (Table III). However, these differences did not account for the excess in neonatal mortality rates. In fact, the sex differential was more prominent in infants born without labor complications.

Age at death. The male excess in neonatal mortality rates was found primarily in deaths occurring within the first week of life (Table IV). Although a slight male excess in neonatal mortality rates occurred after the first week (relative risk = 1.07), it was not statistically significant.

Differences by cause of death. There was a male excess in neonatal mortality rates for almost all reported causes of death (Table V), but it was most pronounced for RDS-HMD. Although these defects are coded separately in the ICDA (776.1, and 776.2) we present them in a combined category because the terms are often used interchangeably (relative risk = 1.57, $p < 10^{-8}$). Not shown in the table are the sex-specific neonatal mortality rates by specific types of infectious diseases, which manifested similar trends (overall relative risk = 1.32, $p < 10^{-8}$). The differences found in sex-specific neonatal mortality rates for congenital anomalies, maternal complications, or neonatal asphyxia were not statistically significant.

Contributions of causes of death to the excess in male neonatal mortality rate. RDS-HMD–related mortality accounted for approximately 43% of the male excess in neonatal mortality rates (Fig. 1). Other individual causes of death contributed less than 10% to the overall male excess. Furthermore, as shown in Table VI, the RDS-HMD–related neonatal mortality rate was greater in male infants with birth weights of 1501 to 2000 gm (relative risk = 2.35, $p < 0.0001$) and 2001 to 2500 gm (relative risk = 3.33, $p < 0.0001$). Within these categories, it accounted for 60% and 56%, respectively, of the male excess in neonatal mortality rates.

Comment

Using population-based data from linked birth and death records, we examined the relationship of known risk factors for neonatal mortality to the well-documented male excess in neonatal mortality. The study has two major findings. First, strong predictors of neonatal death, such as birth weight and maternal obstetric risk, did not explain the sex differential. Moreover, a narrowing of the gap in neonatal mortality rates between sexes tended to occur only for infants at highest risk of neonatal death (such as infants weighing ≤750 gm or born to women with several prenatal risk factors). This finding is consistent with previously reported observations that an overall inverse relationship exists between the infant mortality rate and the male excess in mortality and that the declining rate of infant mortality over the past decades has been accompanied by a relative increase in the male excess in mortality.[8] Such observations have been taken as arguments for an inherent biologic weakness of the male sex, observable only in the absence of other potent environmental or obstetric risk factors for death.[8] As reported by Paneth et al.,[9] the male sex is unique among sociodemographic risk factors in being associated with both increased birth weight and increased neonatal mortality.

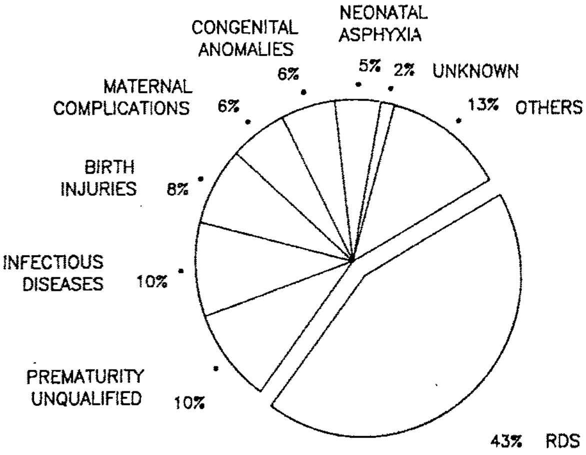

Fig. 1. Contributions of causes of death to the overall excess in male neonatal mortality rate, Georgia neonatal surveillance network, 1974 to 1977.

Second, although the male excess in neonatal mortality rates was noted for several conditions, RDS-HMD–related mortality showed the largest difference between sexes and accounted for almost half of the overall male excess in neonatal mortality rates and almost 60% of the male excess for infants with birth weights of 1501 to 2500 gm. Although this issue has not been examined by previous investigators, we were able to calculate from data available in an earlier study by Naeye et al.[2] that 54% of the male excess in infant deaths in their study was due to RDS.

Despite the limitations of data obtained from vital records, we believe that these do not seriously affect our findings. The ICDA codes for RDS-HMD contain several other diagnostic categories, such as pneumothorax and atelectasis. However, Farrel and Wood[10] found in their review of individual RDS-HMD records that 95% of the cases reported on death certificates were actually RDS. They also reported that RDS-HMD–related mortality was underestimated by 25% when data were obtained from death certificates, since in some instances RDS-HMD was mentioned as a contributing factor but not as the underlying cause of death; however, there is no reason to expect this underascertainment to be different by sex of the infant. In this study, we have information only on the recorded underlying causes of death. Because the gestational ages were unknown in more than 40% of the deaths, we elected not to display the findings. However, the characteristics of the sex differential in mortality by gestational age were similar to that by birth weight, although the relative risks tended to be somewhat smaller (relative risk = 1.39 at 34 to 35 weeks' gestational age).

With these potential limitations borne in mind, the male excess in RDS-HMD–related neonatal mortality could be due to a truly higher incidence of RDS-HMD in male infants, an excess in case-fatality ratio, or both. Several studies have shown, however, that male infants do not have a clinically more severe illness or a higher case-fatality ratio than female infants but do have a higher incidence of RDS.[11, 12] This finding is consistent with studies showing a lag in lung maturation in male fetuses.[13, 14] Finally, recent investigations have shown that antenatal betamethasone administration is more effective in preventing RDS in female than in male infants.[15-17]

In summary, we believe that the findings of these other investigators, coupled with our own, suggest that slower lung maturation among male fetuses is a major contributing factor to the sex differential in neonatal mortality.

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Ultrasound prediction of fetal weight in prolonged pregnancy

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In this study we applied two commonly used birth weight prediction equations to a sample of 121 women with prolonged pregnancies. Subjects had sonographic measurements of biparietal diameter and abdominal perimeter taken within 2 days of delivery at Parkland Memorial Hospital. Although the two prediction equations were obtained from a population of women in New Haven, Connecticut, who delivered over a wide range of gestational ages, when the equations were applied to the sample of prolonged pregnancies in Dallas, Texas, there was a strong correlation (0.71) between predicted and actual birth weight. Moreover, reestimation of the New Haven equations with use of the Dallas data yielded similar regression coefficients. Finally, birth weight prediction equations for black, white, and Hispanic patients in Dallas were not significantly different. These findings suggest a remarkably constant relationship between fetal head and abdominal dimensions and birth weight over different gestational ages and for different population groups. (Am J Obstet Gynecol 1985;151:783-6.)

Key words: Fetal weight, ultrasound, prolonged pregnancy

The estimation of fetal weight in utero plays an important role in the management of a variety of pregnancy complications. Consequently, the accuracy of fetal weight predictions using sonographic measurements has received a great deal of attention.[1-7] Warsof et al.[1] and Shepard et al.[2] have reported two prediction equations that are now in widespread use, which use sonographic measurements of biparietal diameter and abdominal circumference or abdominal perimeter. These equations were obtained by least-squares estimations with the use of data on fetuses whose weights at birth ranged from 174 to 4760 gm.[1]

The use of these equations in birth weight prediction has not been validated for a group of women whose pregnancies are prolonged. Accurate estimation of fetal weight in the case of prolonged pregnancy may be helpful in making decisions concerning the appropriateness, timing, and management of labor induction. However, extrapolation of a least-squares equation to an extreme range of values may be unreliable,[8] and the proportion of fetal subcutaneous fat and other tissue compartments may be altered in prolonged pregnancy. Therefore it may not be appropriate to apply prediction equations that were derived from term or preterm pregnancies to pregnancies that are prolonged. Moreover, it is possible that differences in the demographic characteristics of patient populations in different regions may limit the accuracy of birth weight estimation for one patient population based on an equation that was developed for another population. The present study was undertaken to assess the validity of a widely used birth weight prediction equation that was derived from pregnancies that were predominantly term or preterm in New Haven, Connecticut,[1, 2] as applied to a group of women with prolonged pregnancies who delivered at Parkland Memorial Hospital in Dallas, Texas.

Material and methods

For purposes of this study, women were classified as having certain prolonged pregnancies if (1) they had completed ≥42 weeks according to certain last menses corroborated by fetal heart auscultation between 17 and 20 weeks, (2) fundal height measurements between 20 and 30 weeks were consistent with the last menses, or (3) an ultrasound examination was performed before 26 weeks. Women with unsure menstrual dates, or whose menses were not corroborated as above, were classified as having uncertain prolonged gestations. Women with medical or obstetric complications other than prolonged pregnancy were excluded at enrollment.

From July 11, 1980, to July 8, 1981, 262 women with prolonged pregnancies (76 certain and 186 uncertain) who were being followed in the Obstetric Clinics of Parkland Memorial Hospital underwent an ultrasound examination to assess the volume of amniotic fluid and to estimate fetal weight. Ultrasound findings were not known to clinicians managing these women. Of the 262 women, 121 had an ultrasound examination within 2 days of delivery, 91 of which were within 1 day of delivery. All 121 women with prolonged pregnancies who

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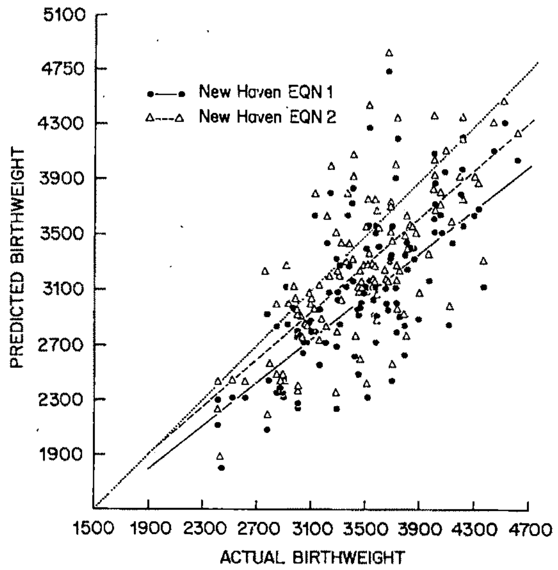

Fig. 1. Predicted versus actual birth weight for New Haven equation 1 and New Haven equation 2. *Dotted line* represents "perfect" prediction.

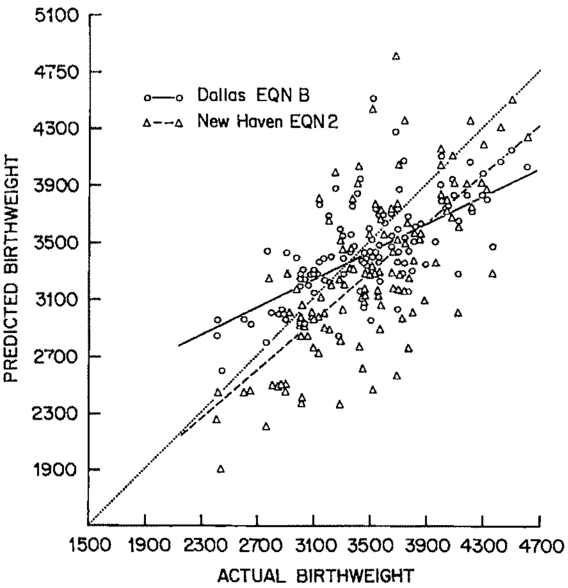

Fig. 2. Predicted versus actual birth weight for Dallas equation B and New Haven equation 2. *Dotted line* represents "perfect" prediction.

underwent sonography within 2 days of delivery were included in the present analysis.

During the ultrasound examination, measurements were taken of the biparietal diameter, head perimeter, and abdominal perimeter. Estimates of head perimeter were made by a graphics analyzer method with use of an electronic digitizer (Numonics Corporation, Lansdale, Pennsylvania). This method was also used to estimate abdominal perimeter. However, abdominal perimeter was also estimated by an ellipse approximation method:

$$AP = \pi(D_1 + D_2)/2$$

where D_1 and D_2 are the major and minor axes, AP is abdominal perimeter, and D is diameter.

Data on gestational age, birth weight, ultrasound examination, and race were entered into a computer file for statistical analysis. Data analysis proceeded in two steps.

First, by use of the following two equations* reported by Warsof et al.[1] and Shepard et al.,[2] predicted birth weights were calculated for each of the pregnancies in our sample:

$$\log_{10}(BW) = -1.599 + 0.144(BPD) + 0.032(AP) - 0.111(BPD^2 \times AP)/1000 \quad (1)$$

$$\log_{10}(BW) = -1.749 + 0.166(BPD) + 0.046(AP) - 2.646(AP \times BPD)/1000 \quad (2)$$

where BW is birth weight and BPD is biparietal diameter. Correlation coefficients were calculated for the relation between actual and predicted birth weight. In addition, differences between actual and predicted birth weight were calculated and used as a further measure of the accuracy of prediction.

The second step of the data analysis was to retain the structure of the two regression equations used by the New Haven group but to reestimate the regression coefficients with use of data from our sample. The coefficients obtained from our data set were then compared with those reported from New Haven. Since head perimeter may be a better measure of head size than biparietal diameter,[7] head perimeter was substituted for biparietal diameter in some equations.

To investigate further the question of whether structural differences exist in the prediction equations for different demographic groups, separate birth weight equations were estimated for black, white, and Hispanic patients in our sample. Differences between the birth weight equations that were obtained for these racial groups were assessed by means of a method developed by Chow,[9] which uses an F statistic to test for equality between sets of coefficients in two linear regressions.

Results

In Fig. 1 actual birth weights in our sample are plotted against predicted birth weights that were computed from both of the New Haven equations. Visually, there is little difference between the prediction equations.

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*Both equations were reported in the first paper from New Haven,[1] but tables for only equation 1 were reported in that paper. Because subsequent experience in New Haven indicated that use of equation 2 led to less underestimation of actual birth weight, a second report[2] gave tables for equation 2.

Table I. Results of regression equations*

Independent variables	Equation					
	A	*B*	*C*	*D*	*E*	*F*
Constant	−1.178	−1.982	−0.281	−0.041	−0.231	−0.265
Biparietal diameter	0.142	0.229†	0.047†		0.047†	0.041†
t statistic	1.58	2.02	5.02		5.01	4.24
Head perimeter				0.004†		
t statistic				2.09		
Abdominal perimeter 1	0.025	0.063†	0.011†	0.013†	0.011†	
t statistic	1.93	1.97	6.71	6.87	6.80	
Abdominal perimeter 2						0.012†
t statistic						6.97
Ultrasound date					−0.004	
t statistic					0.51	
Biparietal diameter^2 × abdominal perimeter/1000	−0.156					
t statistic	1.07					
Biparietal diameter × abdominal perimeter/1000		−5.50				
t statistic		1.61				
Regression coefficient	0.529	0.535	0.524	0.44	0.525	0.531

*Dependent variable = $\log_{10}$ birth weight.
†Statistically significant at 5% level (two-tailed test).

The correlation coefficient between actual and predicted birth weight is 0.706 for New Haven equation 1 and 0.709 for New Haven equation 2.

The difference between actual and predicted birth weights can be defined as the "residual value." In Fig. 1, the residual value is the vertical distance between the observed point and the 45 degree dotted line. The mean of residual values for New Haven equation 1 was −349.7 gm with a standard error of 36.4 gm, and the mean of residuals for New Haven equation 2 was −190.1 gm with a standard error of 37.1 gm. Thus, on average, both equations tended to underestimate actual birth weight, albeit less so for New Haven equation 2.

Estimation of regression equations with use of our data are shown in Table I. Equations A and B show the estimates that resulted when New Haven equations 1 and 2, respectively, were applied to our data. It can be seen from a comparison of equation A with New Haven equation 1 that the two sets of equations are similar:

New Haven equation 1

$$\log_{10} BW = -1.599 + 0.144\ (BPD) + 0.032\ (AP) - 0.111\ (BPD^2 \times AP)/1000$$

Dallas equation A

$$\log_{10} BW = -1.178 + 0.142\ (BPD) + 0.025\ (AP) - 0.156\ (BPD^2 \times AP)/1000$$

Comparison of New Haven equation 2 with Dallas equation B also reveals a strong similarity.

It was not possible to test formally for differences between Dallas and New Haven equations, since this would have required use of the raw New Haven data, which were no longer available in New Haven. However, the similarity in the estimates obtained from the two samples is evident. These results are shown graphically in Fig. 2, which plots the birth weight for each subject in the sample against predicted birth weights as computed from both New Haven equation 2 and Dallas equation B. As noted above, New Haven equation 2 tended to underestimate actual birth weight. By contrast, as actual birth weight increased, Dallas equation B first overestimated and then underestimated birth weight.

Table II. Comparison of regression equations* by race

Independent variable	Race		
	Black (*n* = *49*)	*White* (*n* = *54*)	*Hispanic* (*n* = *18*)
Constant	−0.291	0.239	−0.364
Biparietal diameter	0.048†	0.036†	0.062†
t statistic	3.31	2.30	2.54
Abdominal perimeter 1	0.011†	0.014†	0.010†
t statistic	3.78	6.40	2.13

*Dependent variable = $\log_{10}$ birth weight.
†Statistically significant at 5% level (two-tailed test).

In the process of performing the regression analysis, it was discovered that addition of a term representing the interaction between biparietal diameter and abdominal perimeter, as in equations A and B, reduced the reliability of the regression coefficients. Thus it can be seen from equation C in Table I that when biparietal diameter and abdominal perimeter were entered as the sole independent variables without an interaction term, the t statistics associated with the coefficient estimates of these variables were much higher.

Equation D shows the regression results when head perimeter was substituted for biparietal diameter. This substitution reduced the explanatory power of the model, as reflected by a reduction in the regression coefficient from 0.52 to 0.44.

An attempt was made to determine whether an adjustment should be made for the timing of ultrasound in relation to delivery. This was done by defining a variable, given as the ultrasound date in Table I, which was set equal to 0 or 1 depending on whether the ultrasound examination was performed within 1 or 2 days of delivery, respectively. As can be seen by comparing equations E and C, adjustment for ultrasound date had no impact on the regression coefficients for biparietal diameter and abdominal perimeter.

Equation F shows the results when measurements of abdominal perimeter were taken from the graphics analyzer rather than the ellipse approximation method. Use of the graphics analyzer rather than ellipse approximation to measure abdominal perimeter proved to have no measurable benefit on prediction. There was no statistically significant difference between the coefficients of equation C and equation F with use of the Chow test, and the regression coefficient for the equation as a whole was increased by only 0.007. This is not surprising, since the correlation coefficient between the two methods of measuring abdominal perimeter was 0.91.

Regression equations for black, white, and Hispanic patients are shown in Table II. These equations do not include an interaction term, since it uses up an extra degree of freedom, reduces the reliability of the other regression coefficients, and does not itself enter the regression equation with a statistically significant coefficient. Thus the equation for each racial group reported in Table II has the same structure as equation D for the total population reported in Table I. No differences were observed between the regression equations for the three racial groups (Chow test: black versus white—$F = 0.32$, $p =$ NS; black versus Hispanic—$F = 0.44$, $p =$ NS; black versus Hispanic—$F = 1.03$, $p =$ NS).

Comment

The prediction of birth weight by means of sonographic data, as reported by Warsof et al.[1] and Shepard et al.[2] has enjoyed widespread acceptance. These prediction equations were initially developed and validated on a sample of pregnant women from New Haven, Connecticut, over a wide range of gestational ages. In the present study we report birth weight prediction equations for a sample of patients who were presumed to have prolonged pregnancies. However, these equations should not be construed as definitive prolonged pregnancy equations in view of the high frequency of uncertain dates. In addition, before being put into use, the Dallas equation for prolonged pregnancy would have to be validated on additional samples of women with prolonged pregnancies, both in Dallas and elsewhere. Rather, the main importance of these equations lies in their suggestion that the structural relationship between birth weight and sonographically measured biparietal diameter and abdominal perimeter uncovered in New Haven for a wide range of gestational ages also applies to a group of women with presumed prolonged pregnancies who delivered in Dallas. That is, predicted birth weights for Dallas women by use of New Haven equation 2 were very close to the actual birth weights (although on average there was a slight underestimation). Moreover, reestimation of the prediction equation with use of the Dallas data did not materially improve prediction. Finally, regression equations did not differ significantly among racial groups in Dallas. These findings suggest a virtually constant relationship between fetal head and abdominal dimensions and birth weight for different gestational ages and for different population groups.

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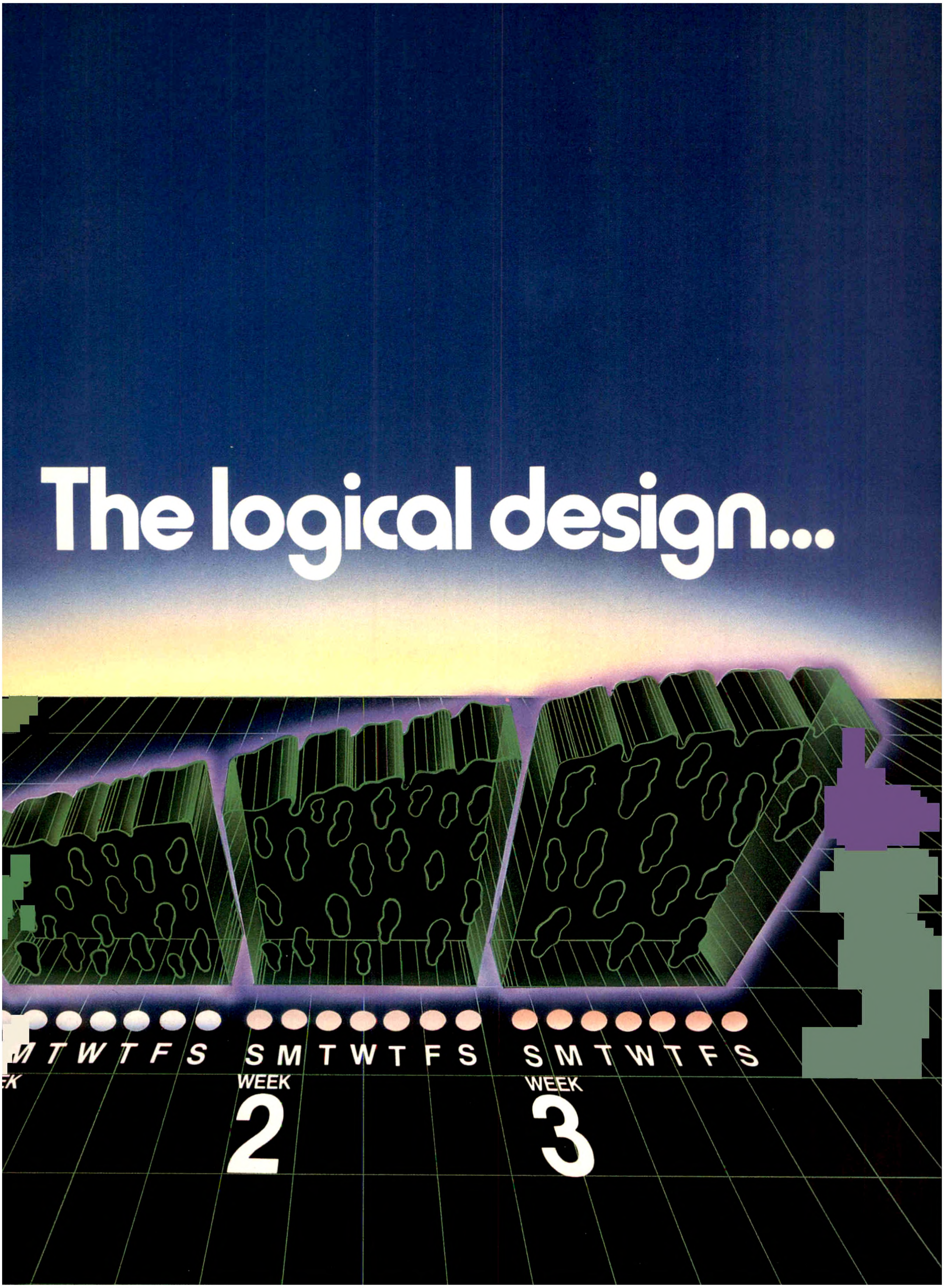
The logical design...
M T W T F S
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The logical choice...

for low breakthrough bleeding[†] in each phase of the cycle

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with week-by-week simplicity

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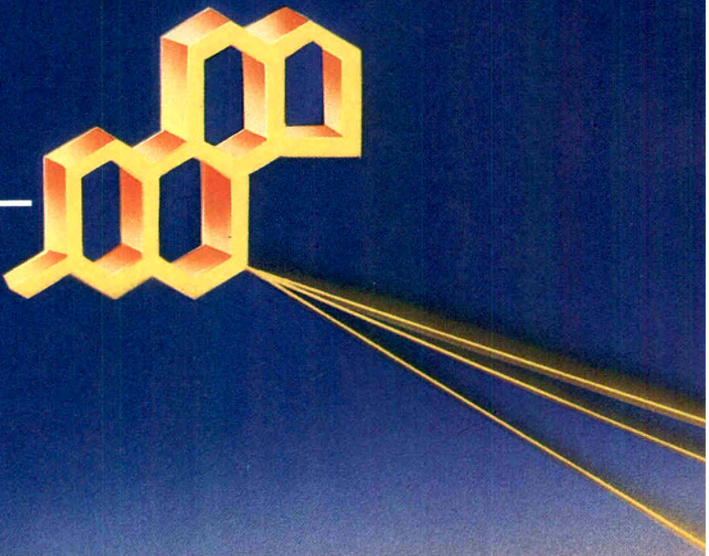
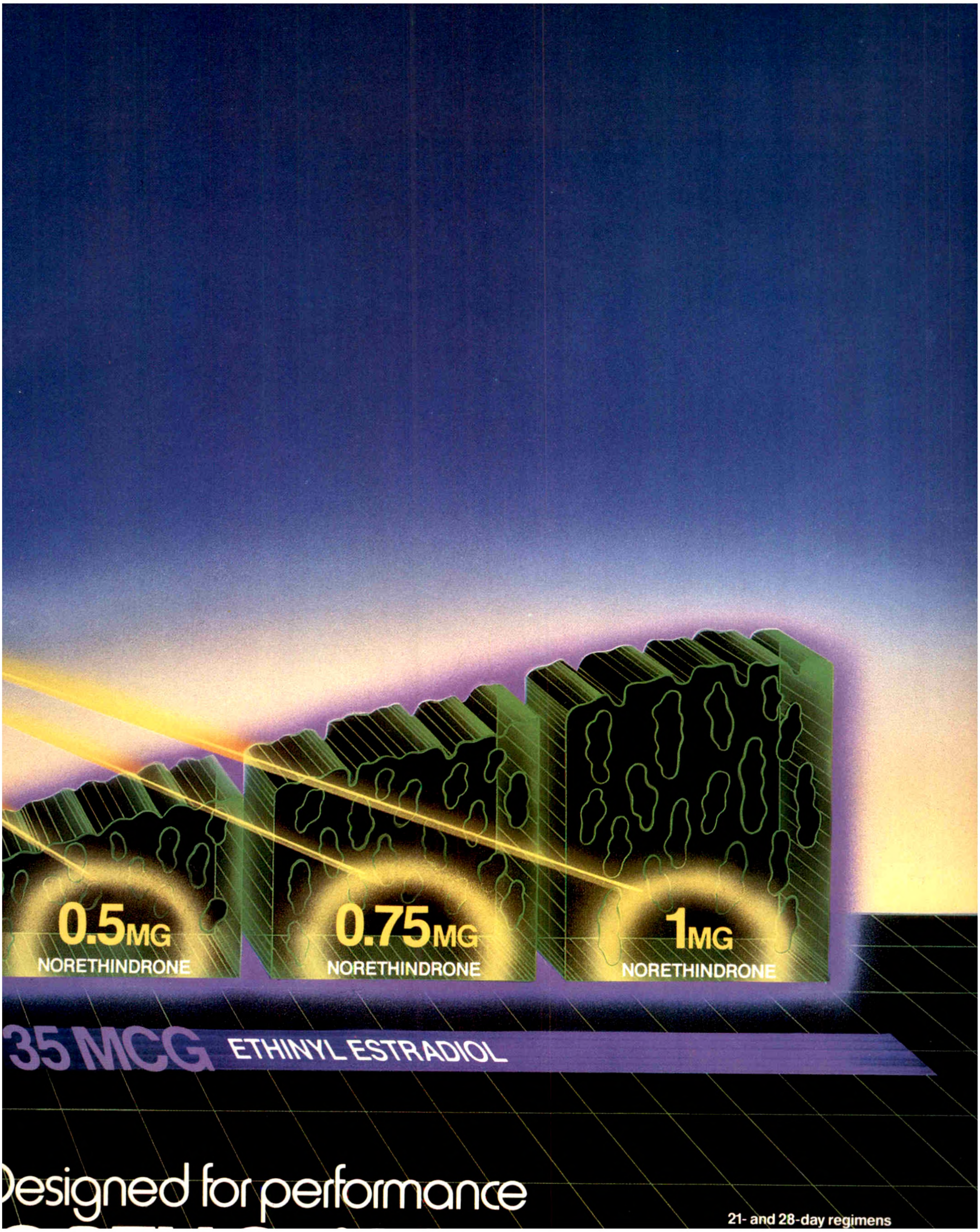
with the progestogen– norethindrone



[†] Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a summary of which follows. © OPC 1985 OJ-482


0.5MG
NORETHINDRONE
0.75MG
NORETHINDRONE
1MG
NORETHINDRONE
35 MCG ETHINYL ESTRADIOL
Designed for performance
21- and 28-day regimens

ORTHO-NOVUM* 7/7/7 Tablets (norethindrone/ethinyl estradiol)

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.
DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.
CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

• ORTHO

Soft drug use after pregnancy compared to use before and during pregnancy

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With the use of information gathered through the course of pregnancy and data collected after delivery, the changing patterns of alcohol, nicotine, and marijuana use of 288 women participating in the Ottawa Prenatal Prospective Study were investigated. The time periods considered were the year before pregnancy, each trimester of pregnancy, and 1, 6, and 12 months post partum. Alcohol use showed the most marked reduction of the three drugs during pregnancy, but a year after delivery prepregnancy drinking patterns (including binging) had been reestablished. An exception to this was a continued reduction of alcohol consumption by those categorized as heavy social drinkers prior to pregnancy. Although not as marked as the alcohol reduction, nicotine use was reduced during pregnancy and continued to remain at the reduced level 1 year post partum. Heavy marijuana use was the least reduced of the drugs during pregnancy and consumption returned to prepregnancy levels a year after the birth of the baby. (AM J OBSTET GYNECOL 1985;151:787-92.)

Key words: Alcohol, nicotine, marijuana, pregnancy, drug use

Among North American women, "soft" drug use decreases during pregnancy.[1-3] However, there are virtually no data addressing the issue of whether patterns of drug use after pregnancy reflect prepregnancy habits or continue at the lower levels of consumption reached during pregnancy.[3]

The issue of postnatal "soft" drug use raises several important issues. For the mother, a reduction in cigarette, alcohol, or marijuana consumption is directly beneficial to her health. The likelihood that a reduction in drug use during pregnancy will continue after the birth of the baby may be used as a further argument in persuading the mother-to-be to reduce her "soft" drug use during pregnancy. Conversely, if there is a high probability of postpartum drug use reverting to prepregnancy levels, appropriate counseling during pregnancy or immediately after birth to encourage women to continue the newly developed reduced habits would be appropriate.

This report examines patterns of "soft" drug use up to a year after the birth of the baby and compares these patterns to habits before and during pregnancy.

Methods

In 1978, a collaborative study involving the Department of Psychology at Carleton University and the major hospitals in the Ottawa area was initiated. Subjects volunteered to participate during their pregnancy after being informed of the research either by their obstetrician or by notices in the media. The purpose of this ongoing work is to determine the patterns of marijuana use and the levels of alcohol and cigarette use during pregnancy and to examine, in a prospective fashion, the effects of these drugs on the offspring. Details of the procedures and epidemiologic information have been presented elsewhere.[1]

In January, 1983, a 19-page questionnaire booklet was sent to all women participating in the Ottawa Prenatal Prospective Study whose offspring were at least 1 year of age by that date. The portion of the questionnaire dealing with life-style habits is discussed in the present report. Information was gathered retrospectively about the pattern and quantity of alcohol consumption and tobacco, marijuana, and hashish use, at 1 month, 6 months, and 1 year after the birth of the child. Demographic and drug use information pertaining to the year before pregnancy and each trimester had been collected previously as part of the interviews conducted during pregnancy.[1]

The categorization of drugs has been described in detail by Fried et al.[1] Alcohol consumption was broken down into beer, wine, and liquor, with both quantity and pattern of consumption being noted and then converted to average ounces of absolute alcohol per day. Four categories of alcohol consumption were developed: abstainers, light (<0.14 ounces of absolute alcohol per day), moderate (0.14 to 0.85 ounces of absolute alcohol per day), and heavy (>0.85 ounces of absolute alcohol per day). Approximately 0.85 ounces of absolute alcohol would be contained in 1½ bottles of regular Canadian beer or two 4-ounce glasses of wine

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Table I. χ^2 Values (Stuart-Maxwell statistic[18]) based on comparisons of proportion of women within drug categories during various time periods

	Drug		
Time period	*Alcohol (df = 3)*	*Nicotine (df = 2)*	*Marijuana (df = 3)*
Prepregnancy by first trimester	118.83*	21.24*	25.66*
Prepregnancy by third trimester	118.70*	32.11*	36.92*
Prepregnancy by 12 months post partum	38.93*	15.49*	22.42*
First trimester by third trimester	9.05†	7.39†	13.02‡
Third trimester by twelve months post partum	51.45*	14.49*	4.84
One month post partum by 12 months post partum	53.06*	19.28*	16.59†

*$p < 0.001$.
†$p < 0.05$.
‡$p < 0.01$.

or two drinks containing distilled alcohol. Binge drinking was defined according to the categories of alcohol use described above and procedures summarized by Streissguth et al.[4] Three binge-drinking patterns were considered: (1) low volume, high maximum—<0.14 ounce of absolute alcohol per day and more than five drinks per occasion; (2) medium volume, high maximum—0.14 to 0.85 ounce of absolute alcohol per day and more than five drinks per occasion; (3) high volume, high maximum—>0.85 ounce of absolute alcohol per day and more than five drinks per occasion.

The amount of nicotine consumed was calculated by multiplying the number of cigarettes smoked by the nicotine content of the brand smoked. Three categories were used: nonsmokers, light smokers (<16 mg of nicotine per day), and heavy smokers (>16 mg of nicotine per day). Marijuana users were classified into four groups: nonusers, light users (less than one joint per week or exposed to exhaled marijuana smoke of others), moderate users (two to five joints per week), and heavy users (more than five joints per week). If hashish use was reported, the number of joints was multiplied by five to take into account the estimated greater concentration of tetrahydrocannabinol in the latter substance.[5]

Results

Of the 371 postnatal questionnaires distributed, 288 (78%) were received by the cutoff date in May, 1983. The demographic characteristics of the women were highly similar to those found in samples described elsewhere.[1,6] The women were predominantly middle-class with an average family income of $31,247 and with more than 96% completing high school and 57% having graduated from a postsecondary institution.

With the data gathered in the interviews conducted during pregnancy it was possible to compare the responders and nonresponders. The two samples were very similar in terms of age, family income, parity, and education. The extent of use of alcohol and nicotine during pregnancy was indistinguishable between the two groups of women. Although the difference was not statistically significant, the extent of heavy marijuana use was greater among the nonresponders (9% prior to pregnancy and 7% in the third trimester) compared to the responders (5% prior to pregnancy and 3% in the third trimester).

Once pregnancy was established, there was a significant reduction in alcohol intake which continued until the third trimester (Table I). Following the birth, alcohol consumption increased and continued to do so for 12 months post partum. As seen in Fig. 1, before pregnancy the greatest proportion of women were moderate alcohol users, while most women were light users from the first trimester to 1 month after delivery. By 12 months after delivery, most women were once again in the moderate category of alcohol consumption.

The average daily consumption of the 52 women who were categorized as heavy social drinkers prior to pregnancy was 1.70 ounces of absolute alcohol. Of these women, 37 were no longer heavy social drinkers a year after delivery, consuming an average of 0.50 ounce of absolute alcohol per day. The 15 women who were still categorized as heavy social drinkers 1 year after delivery had a prepregnancy daily average of 1.75 ounces of absolute alcohol. During pregnancy their drinking remained essentially unchanged and a year after delivery these women consumed an average of 1.50 ounces of absolute alcohol per day. Table I shows the changing proportion of women within each alcohol category in the various time periods.

Patterns of drinking from before pregnancy to 1 year after pregnancy were also examined in terms of consumption of beer, wine, and distilled beverages.

Before pregnancy 57% of the sample drank beer, 92% drank wine, and 86% drank various forms of distilled alcoholic beverages. By the third trimester only 50% of prepregnancy beer drinkers continued to drink

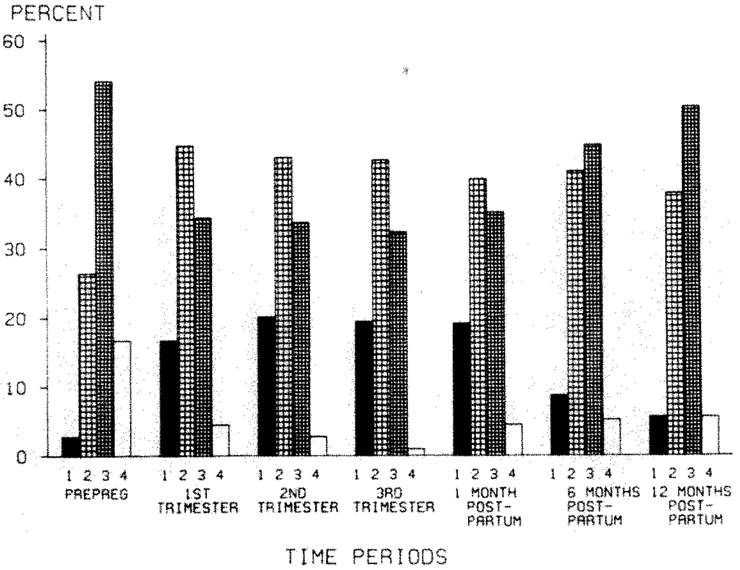

Fig. 1. Categories of alcohol use during seven time periods (n = 288). *1* = Abstainers; *2* = light users; *3* = moderate users; *4* = heavy users.

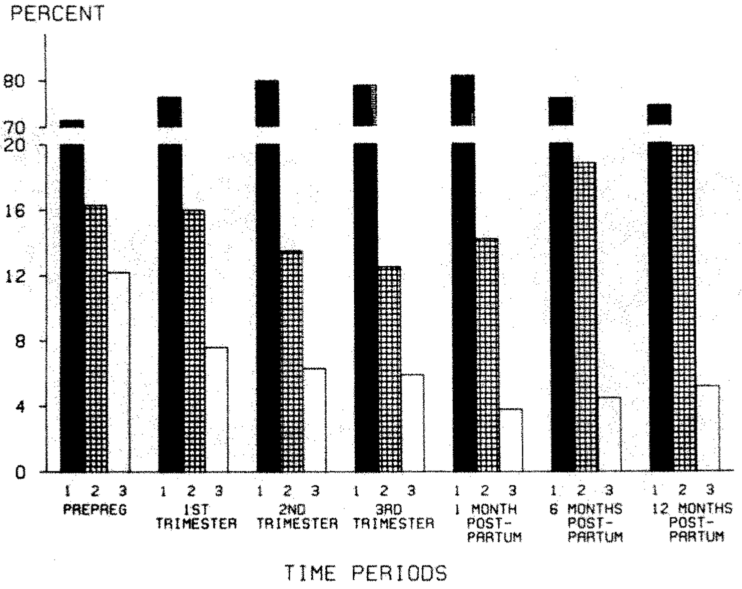

Fig. 2. Categories of nicotine use during seven time periods (n = 288). *1* = Abstainers; *2* = light users; *3* = heavy users.

that beverage and only 43% of those who drank distilled alcoholic beverages continued to do so. In contrast, of the women who drank wine prior to pregnancy 73% were still doing so by the third trimester. By the year after pregnancy the proportion of beer and wine drinkers had returned to within 3% of prepregnancy figures while the proportion of women drinking distilled beverages was 11% less than the number doing so prior to pregnancy.

Before pregnancy beer accounted for 27.2% of the total ounces of absolute alcohol consumed, wine 44.5%, and distilled alcohol 28.3%. During and after pregnancy these relative percentages remained constant.

An examination of binge drinking by the 288 women revealed that 28% reported binging before pregnancy. There was a sharp decline in binging throughout pregnancy with 9% doing so in the first trimester (compared to before pregnancy, McNemar test $X = 53.01$, $p < 0.001$) and only 1% in the third trimester (compared to first trimester, $X = 76.01$, $p < 0.001$). After the birth there was a significant increase in binging with 9% doing so at 1 month (compared to the third trimester,

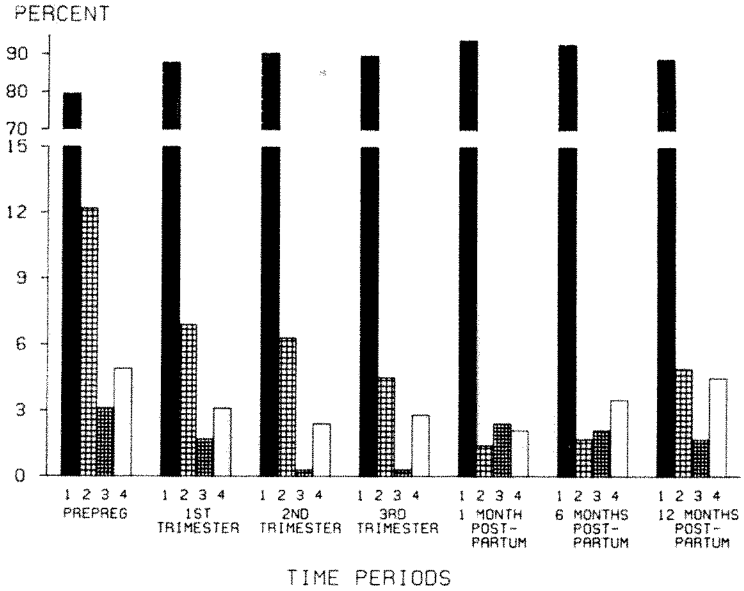

Fig. 3. Categories of marijuana use during seven time periods (n = 288). *1* = Abstainers; *2* = light users; *3* = moderate users; *4* = heavy users.

X = 21.04, $p < 0.001$), 16% at 6 months (compared to 1 month post partum, X = 17.05, $p < 0.001$), and 21% at 12 months (compared to 6 months, X = 16.06, $p < 0.001$). When binging did occur, approximately two thirds of the women did so with only one type of alcoholic beverage, with wine being most frequently consumed. Before pregnancy approximately 70% of the women who binged were in the medium alcohol, high maximum category, 20% were in the high alcohol, high maximum category, and only 10% were in the low alcohol, high maximum category.

Although not as marked as changing alcohol patterns, nicotine consumption also declined once pregnancy was determined (Fig. 2). Cigarette use continued to decline during pregnancy and, unlike alcohol, continued to decrease during the first month after birth. There was only a slight increase in the number of heavy smokers by 6 months post partum. One year after the birth, the proportion of women categorized as heavy smokers had increased their smoking only to the level of that observed in the third trimester of pregnancy.

The changing patterns of marijuana use during and after pregnancy (Fig. 3) were somewhat different than those observed with either alcohol or nicotine. Once pregnancy was determined the number of women reporting irregular or light use (less than one joint per week) decreased from a prepregnancy rate of 12% to 7% in the first trimester and 4.5% in the third trimester. A similar decrease was observed among the moderate users (two to five joints per week). However, the proportion of heavy users decreased only slightly from 5% to 3% between the prepregnancy period and the first trimester and remained relatively constant as pregnancy proceeded. One year after the birth the proportion of heavy users had returned to prepregnancy levels but the number of light and moderate users remained at the low levels seen during the third trimester.

Comment

The data clearly indicate that the patterns of use of the three "soft" drugs under investigation differ over the prepregnancy, pregnancy, and postpartum time periods being considered. As the patterns of the "soft" drugs in question have remained very consistent between 1978 and 1982,[6] the shifts in usage described in this report are not due to changing general trends in drug consumption among nonpregnant women.

By 12 months post partum, most prenatal drinking patterns, including the type of beverage drunk most often (wine), the relative contribution that beer, wine, and distilled products make to the total amount of alcohol consumed, and the frequency of binge drinking, have been reestablished. An exception to this reversion was found in those women categorized as heavy social drinkers prior to pregnancy. Seventy-one percent of these women continued their reduction in alcohol consumption during pregnancy at 1 year post partum. This is a valuable and unexpected benefit of educational campaigns directed at encouraging women to moderate their drinking during pregnancy.

The relatively low number of beer drinkers in all seven time periods considered is consistent with the observation made by Streissguth et al.[4] that beer was consumed more often by those who were more poorly educated and by those in lower social classes. In the present study the sample was composed of predominantly middle-class women. It is noteworthy that two studies have linked beer (more than other types of alcoholic beverages) with adverse pregnancy outcomes.[7,8] The association between socioeconomic risk factors and the consumption of a type of alcoholic beverage that may particularly increase risk is a combination that deserves special attention. Binge drinking was found among all categories of social drinkers. Because of the potential risk associated with bursts of drinking,[9,10] care must be taken to advise pregnant women who drink any amount of alcohol about this aspect of their alcohol consumption.

During pregnancy the increase in the number of nonsmokers was not as marked as the increase of alcohol abstainers. However, in the first trimester compared to the prepregnancy period, there was a tendency among the heavy smokers to reduce their nicotine consumption as opposed to ceasing smoking entirely. As the risk posed by cigarette use during pregnancy is clearly dose-dependent,[11] any reduction in nicotine use is of benefit to the fetus. As such, the success or failure of programs designed to encourage pregnant women to alter their smoking habits must not be evaluated solely in terms of the number of women who give up cigarettes entirely.

One of the most striking findings of the present study was that the average reduction in nicotine use seen during pregnancy continued into the first month after pregnancy, and 1 year after the birth of the baby use was very similar to that seen in the third trimester. The reasons for the trend of continued reduction of nicotine consumption in the first 4 weeks after delivery may reflect, in part, a concern among women about the effects of smoking on breast-feeding. That the decline in smoking associated with pregnancy lasts for at least a year is a very encouraging finding. It has obvious health benefits for the woman. Further, knowing that the probability of a reduction during pregnancy will likely persist beyond the birth of the baby provides additional motivation for the smoker to reduce her habit prior to becoming pregnant.

The heavy marijuana users (more than five joints per week) demonstrated the least alteration of their pattern of use either during pregnancy or after delivery. It has previously been shown that among the women participating in the Ottawa Prenatal Prospective Study[1,6] marijuana use is associated with a lower socioeconomic level and less formal education. Possibly women with these demographic characteristics might be less likely to alter their prepregnancy life-style habits. Other workers[8] have noted that, among alcohol consumers, women who altered their drinking habits the most during pregnancy were the most highly educated. An additional factor that may be contributing to the pattern of use seen among the heavy marijuana users may be that the potential risk factors associated with marijuana, unlike alcohol or cigarettes, and human pregnancy are only just beginning to appear in the scientific literature.[12-16] As a result, compared to the other drugs discussed, very little professional attention or media activity has been directed at encouraging women to reduce their marijuana habits during pregnancy.

The women in the present study were all volunteers and have been accustomed to questions regarding their drug intake. These factors plus the rapport that was established during the repeated pregnancy interviews and testing of offspring[1,13] served to increase the likelihood of the validity of the subjects' responses.[16] It is unlikely that recall bias confounded the results. The patterns of drug use during pregnancy were determined prior to the birth and thus the possibility of underreporting by women who had a poor pregnancy outcome[17] was precluded by the timing of the interviews. As the postnatal effects of "soft" drug use have not had the same publicity as the prenatal effects, a systematic recall bias operating in one direction for one of the drugs and in another direction for the other drugs appears unlikely.

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Steroid metabolism by human chorion laeve from dichorionic twin pregnancies

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Certain steroid metabolic properties of chorion laeve from dichorionic twin pregnancies were examined to determine whether they were present in chorion not contaminated by decidua or serum. In the chorion situated between the two amniotic sacs and not in contact with decidua, aryl sulfatase, 3β-hydroxysteroid dehydrogenase, and aromatase activities were found. This indicates that these reactions are present in chorion laeve and were not previously ascribed to this tissue because of decidual contamination. Specific cortisol binding was also present in this area of chorion laeve, which excludes serum contamination. It is suggested that the specific steroid-binding protein in the membranes may be derived from the transcortin-like protein present in amniotic fluid. (AM J OBSTET GYNECOL 1985;151:792-5.)

Key words: Steroid metabolism, cortisol binding, chorion laeve, dichorionic twin pregnancies

Human fetal membranes and decidua may play an important role in the onset of human labor because of their position in the uterus and because they are able to synthesize and metabolize prostaglandins.[1-3] The tissues have also been shown to metabolize steroids to varying degrees and it has been proposed that the local action of steroids in these tissues may play a role in the onset of labor.[4-6]

One of the difficulties encountered in studies with the outer membrane, the chorion laeve, has been that of obtaining pure tissue which is not contaminated by maternal decidua. In fact, it has been suggested that the 11β-hydroxysteroid dehydrogenase activity in fetal membranes, originally assigned to the chorion, is really of decidual origin.[7] In this study the 11β-hydroxysteroid dehydrogenase activity was not detected in the chorion laeve from dichorionic twin pregnancies which was not in contact with decidua, that is, the area of the chorion which forms the wall between the two amniotic cavities. In the present study we have examined whether other steroid-metabolizing properties previously assigned to the chorion laeve are present in the chorion that is not in contact with decidua. Specifically, the 3β-hydroxysteroid dehydrogenase, aryl sulfatase, and aromatase activities were examined.

A specific steroid-binding protein has previously been described in chorion laeve and amnion,[4, 8] but because of its similarity to transcortin one cannot exclude that its presence in the chorion laeve is not due to serum contamination. In dichorionic twins the chorion laeve in the wall between the two amniotic sacs is fused and cannot be contaminated by serum; therefore, we examined whether or not this tissue contained the specific steroid binding.

Material and methods

Materials. Steroids were purified by thin-layer chromatography and crystallization before use. Nucleotides were purchased from Sigma Chemical Co., St. Louis, Missouri; [7-n^{3}H]testosterone (specific activity, 8.9 Ci/mmol) and [7-n^{3}H]dehydroepiandrosterone (specific

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Table I. Enzyme activities in chorion laeve from dichorionic twins

Tissue	Enzyme	Enzyme activities	
		Fused chorion	Uterine chorion
1	Sulfatase (nmol/hr/mg protein)	0.75	ND
	3β-Hydroxysteroid dehydrogenase (pmol/min/mg protein)	246	283
	Aromatase (pmol/hr/mg protein)	0.031	ND
2	Sulfatase (nmol/hr/mg protein)	1.64	0.7
	3β-Hydroxysteroid dehydrogenase (pmol/min/mg protein)	45	44
	Aromatase (pmol/hr/mg protein)	0.052	0.036
3	Sulfatase (nmol/hr/mg protein)	0.84	0.40
	3β-Hydroxysteroid dehydrogenase (pmol/min/mg protein)	156	200
	Aromatase (pmol/hr/mg protein)	0.12	0.15

ND = Not detectable. Each value is the mean of duplicate determinations.

activity, 23 Ci/mmol) from Amersham, Oakville, Ontario; and [7-n^{3}H]dehydroepiandrosterone sulfate ammonium salt (specific activity, 24 Ci/mmol) and [1,2,6,7,^{3}H]cortisol (specific activity, 93.1 Ci/mmol) from New England Nuclear, Boston, Massachusetts. Decolorizing charcoal (Norit A) and dextran T70 were purchased from J. T. Baker Chemical Co., Phillipsburg, New Jersey, and Pharmacia Fine Chemicals, Uppsala, Sweden, respectively. All reagents used were of the American Chemical Society specifications or the best commercial material available. Thin-layer chromatography was carried out with 10 by 10 cm plates of silica gel GHLF purchased from Analtech, Newark, Delaware.

Methods. Tissues were obtained at 38 to 40 weeks' gestation, prior to elective cesarean section, or after spontaneous vaginal delivery. They were immediately placed on ice and transported to the laboratory. All further manipulations were carried out at 4° C.

The membranes and attached decidua were removed by cutting the tissues 1 inch above the placental plate. Amnion was separated from the chorion laeve, and decidua attached to the chorion was carefully removed by a scalpel. The chorion laeve between the two amniotic sacs is referred to as fused chorion and that in contact with decidua as uterine chorion. Tissue homogenates were prepared as previously described.[8] Homogenates were centrifuged at 800 × g for 10 minutes and the resulting supernatant was centrifuged at 105,000 × g for 60 minutes. The particulate fraction which sedimented at 105,000 × g was suspended in 0.05 mol/L of monosodium-disodium phosphate buffer, pH 7.4, containing 0.25 mol/L of sucrose and 7 mmol/L of β-mercaptoethanol, and recentrifuged at 105,000 × g for 60 minutes. This particulate fraction was then stored frozen at −60° C and used for all enzyme assays which were carried out on the thawed fractions within 10 to 14 days of freezing.

Steroid sulfatase assay. The reaction was carried out in a total volume of 0.5 ml in 0.05 mol/L of monosodium-disodium phosphate buffer, pH 7.4, containing 7 mmol/L of β-mercaptoethanol and 0.25 mol/L of sucrose. The substrate, 4 μmol/L of [7-n^{3}H]dehydroepiandrosterone sulfate, was preincubated in the reaction buffer with shaking at 37° C for 5 minutes. The reaction was started by the addition of an appropriate volume of membrane fraction and was allowed to proceed for 1 hour at 37° C. Ethanol (0.1 ml) containing nonradioactive carriers (dehydroepiandrosterone and dehydroepiandrosterone sulfate, 10 μg each) and [4-^{14}C]dehydroepiandrosterone as a recovery standard was added, and the reaction medium immediately extracted three times with two volumes of benzene. The combined benzene extracts were then washed with half a volume of water; the mixture was centrifuged and the benzene layer removed. The washed extract was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 80 μl of benzene and a 20 μl aliquot was chromatographed on silica gel plates (chloroform : acetone, 4 : 1, v/v). The radioactivity corresponding to dehydroepiandrosterone was quantitated with the ^{14}C-labeled steroid used as a recovery standard. In the control vessels the membrane suspension was omitted and the vessels were extracted and counted as described above. This amount was subtracted from the reaction values.

3β-Hydroxysteroid dehydrogenase assay. This assay was carried out under conditions similar to those described previously[9] with the following changes. The total reaction volume was 0.5 ml. The concentration of [7-n^{3}H]dehydroepiandrosterone was 100 nmol/L and that of nicotinamide adenine dinucleotide was 1 mmol/L. Reactions were carried out for 5 minutes. Extraction and two-dimensional chromatography were carried out as described for the aromatase.[10] The only steroid product found under these conditions was androstenedione and the rate of conversion of substrate to this steroid was determined with [4-^{14}C]androstenedione used as the recovery standard.

Aromatase assay. This assay was carried out under

Table II. Recrystallization of steroids formed by the fused chorion from dichorionic twins

Reaction	*Product isolated*	*Solvent system*	*^3H:^{14}C ratio*
Aromatase	17β-Estradiol	Initial*	3.10
		Benzene:ethanol (9 : 1, v/v)	3.15
		Diethyl ether	3.07
3β-Hydroxysteroid dehydrogenase	Androstenedione	Initial*	15.9
		Acetone:water (1 : 1, v/v)	15.8
		n-Hexane:ethanol (4 : 1, v/v)	15.1
Sulfatase	Dehydroepiandrosterone	Initial*	9.8
		Acetone:water (1 : 1, v/v)	9.4
		n-Hexane:ethyl acetate (5 : 1, v/v)	9.5

*This is the initial ^{3}H:^{14}C ratio of the area on the chromatogram corresponding to this steroid.

Table III. Specific cortisol binding in chorion laeve from dichorionic twins

	Specific binding (mean ± SEM) (fmol · mg protein^{-1})	
Tissue	*Fused chorion*	*Uterine chorion*
1	2056 ± 175	2475 ± 307
2	1796 ± 261	463 ± 54

Each value is the mean ± SEM of triplicate determinations.

conditions similar to those previously described[10, 11] with the following alterations. The total reaction volume was 0.5 ml. The concentration of [7-n^{3}H]testosterone used was 7 nmol/L and the reaction was carried out for 3 hours at 37° C. The reduced nicotinamide dinucleotide phosphate concentration was 1.0 mmol/L, and 10 μl of a 50 mmol/L solution of reduced nicotinamide dinucleotide phosphate was added each 30 minutes. Extraction of the reaction medium, two-dimensional thin-layer chromatography, and quantitation of the conversion to estrogens were carried out as previously described, with [4-^{14}C]estradiol used as the recovery standard.

Specific cortisol binding. This was determined with 10 nmol/L of cortisol in the 105,000 × g supernatant fraction which was pretreated with dextran-coated charcoal to remove endogenous steroids, as previously described.[8]

Radioactive counting. The radioactivity was quantitated with a Beckman LS 7500 counter and 10 ml of Beckman Ready-Solve MP used as the scintillant. The efficiency for tritium was about 35% and that of [^{14}C] 73%.

Protein determination. Protein was assayed by the method of Lowry et al.[12] with the modification of Hartree.[13]

Results

Previous studies have shown that the sulfatase,[14] 3β-hydroxysteroid dehydrogenase,[5] and aromatase activities[11] in the membranes were present mainly in the subcellular fractions sedimenting at 10,000 and 105,000 × g. In contrast, the specific binding of cortisol was found in the soluble protein fraction (105,000 × g supernatant). Therefore, the subcellular membrane fraction was used to measure the enzyme activities and the 105,000 × g supernatant to measure the specific cortisol binding.

In the three tissues examined, sulfatase, 3β-hydroxysteroid dehydrogenase, and aromatase activities were found in the fused chorion (Table I). Even in tissue No. 1 in which no sulfatase or aromatase activity was detected in the chorion in contact with the uterus the fused chorion had substantial activity. The identity of the products of each of the reactions in the fused chorion was confirmed by crystallization to a constant tritium : carbon 14 ratio (Table II).

The results obtained for the specific binding of cortisol in the two areas of the chorion are shown in Table III. In the fused chorion compared to the uterine chorion, similar or higher levels of specific cortisol binding were found.

Comment

The results presented indicate that sulfatase, 3β-hydroxysteroid dehydrogenase, and aromatase activities are present in chorion laeve, and their presence in the tissue is not due to decidual contamination. This contrasts with what was found with the 11β-hydroxysteroid dehydrogenase.[7] This enzyme was not found in the fused chorion and thus the authors proposed that previous reports of its presence in chorion laeve were due to decidual contamination. The present study reinforces the 11β-hydroxysteroid dehydrogenase study as it shows that the fused chorion is metabolically active and not degenerated. These results would also suggest that the hormonal and nutritional supply derived from the amniotic fluid can adequately maintain the chorion at least with respect to the metabolic functions examined.

Previously we have used amnion to study the specific steroid-binding protein associated with human fetal membranes in order to circumvent possible interference by serum transcortin. In this study we have shown that the fused chorion, which is not contaminated with serum, contains the specific steroid-binding protein. The presence of the specific binding protein in this area of chorion might also suggest that it is the same as the transcortin-like protein found in amniotic fluid.[15] If this were the case the increase in the protein concentration in term membranes[4] may be indicative of glucocorticoid and/or progesterone movement into the membranes. Recent studies by our laboratory indicating that the specific cortisol-binding protein in the membranes may be extracellular[16] would also be consistent with this hypothesis.

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High long-standing fertilizing capacity of human sperm isolated for male sex preselection

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A method of separating a Y-enriched human sperm population for male sex preselection with a single-column, four-step, four-layer albumin gradient technique is presented. This technique provides an efficient separation of spermatozoa, good Y-enriched sperm motility, and high fertilization capacity of this fraction as tested by the zona-free hamster ova sperm penetration assay. (AM J OBSTET GYNECOL 1985;151:795-8.)

Key words: Sex preselection, Y human sperm traction, fertilization, zona-free hamster penetration assay

A method of separating Y-enriched human sperm populations for male sex preselection was evaluated. The satisfactory Y-enrichment of the sperm fractions that was obtained and their excellent viability, motility, and high fertilizing capacity support the use of the method for clinical purposes. The possibility of sex preselection might be a valuable contribution in the prevention of sex-linked genetic diseases.

Concentration gradients of human serum albumin have been used for Y-enrichment of sperm populations, to be later employed for sex preselection; how-

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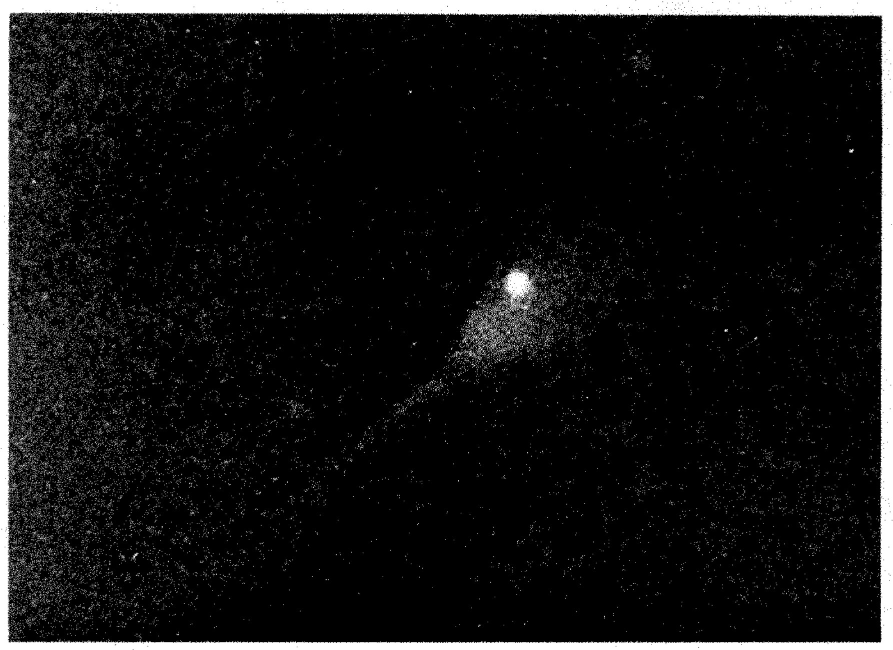

Fig. 1. The fluorescent Y body is clearly seen within the spermatozoon (Y-spermatozoon) following staining with quinacrine dihydrochloride, with use of ultraviolet light. (× 11,000.)

Table I. Separation of human spermatozoa by albumin gradients

	Before separation					*After separation*				
	X sperm	*Y sperm*	*Motility*	*Penetration*	*Sperm per penetrated ovum ratio*	*X sperm*	*Y sperm*	*Motility*	*Penetration*	*Sperm per penetrated ovum ratio*
n	12	12	12	12	12	12	12	12	12	12
Mean	56.2	43.8	75.6	54.1	1.3	22.6	77.4	96.2	100	4.7
±SE* (%)	±3.7	±3.7	±6.4	±6.1	±0.2	±4.3	±4.3	±3.3	±0	±0.6
Range (%)	53-64	36-47	68-90	25-70	1-3	18-34	66-82	92-100	100-100	2-8

*Mean value ± standard error.

ever, insemination by means of Y-enriched sperm resulted in low conception rates.[1]

In the present experiment, sperm isolated for male preselection was tested for its penetration capacity into zona-free hamster ova prior to clinical trial. We employed a single-column four-step four-layer albumin gradient technique for sperm isolation, which is a modification of the two-column three-step three-layer method first demonstrated by Beernink and Ericsson.[2]

The separation technique used in this study commences with the preparation of a four-layer albumin gradient in a 120 × 16 mm plastic column. The four different concentrations of salt-poor human serum albumin are layered on the column in the following order (from bottom to top): 20%, 12.5%, 8.3%, and 4%. The liquefied ejaculate is then diluted by Tyrode's solution (1 : 1 v/v) and the diluted specimen is divided into 0.8 ml aliquots of washed sperm which are layered on top of the column. Each column does not contain more than 5×10^7 cells. The initial sperm layer is discarded 45 minutes after it has been added. At intervals of 30, 15, and 30 minutes, the 4%, 8.3%, and 12.5% human serum albumin layers, respectively, are removed. Total time of the separation procedure on the column is therefore 2 hours. The 20% human serum albumin layer is then centrifuged at 600 g for 10 minutes and the sperm-disc resuspended in 0.2 ml Tyrode's solution and tested for its penetration capacity by means of the in vitro hamster ova penetration assay. This assay has been proved more sensitive than motility assessment in predicting the fertilizing capacity of human spermatozoa. Briefly, in this assay tubal hamster oocytes, previously treated with hyaluronidase (1 mg/ml) and trypsin (1 mg/ml) to remove the cumulus and zona pellucida, respectively, are interacted with washed and preincubated human spermatozoa. After 3 hours of sperm-ova interaction, the ova are removed from the insemination dishes, washed, fixed, stained with ace-

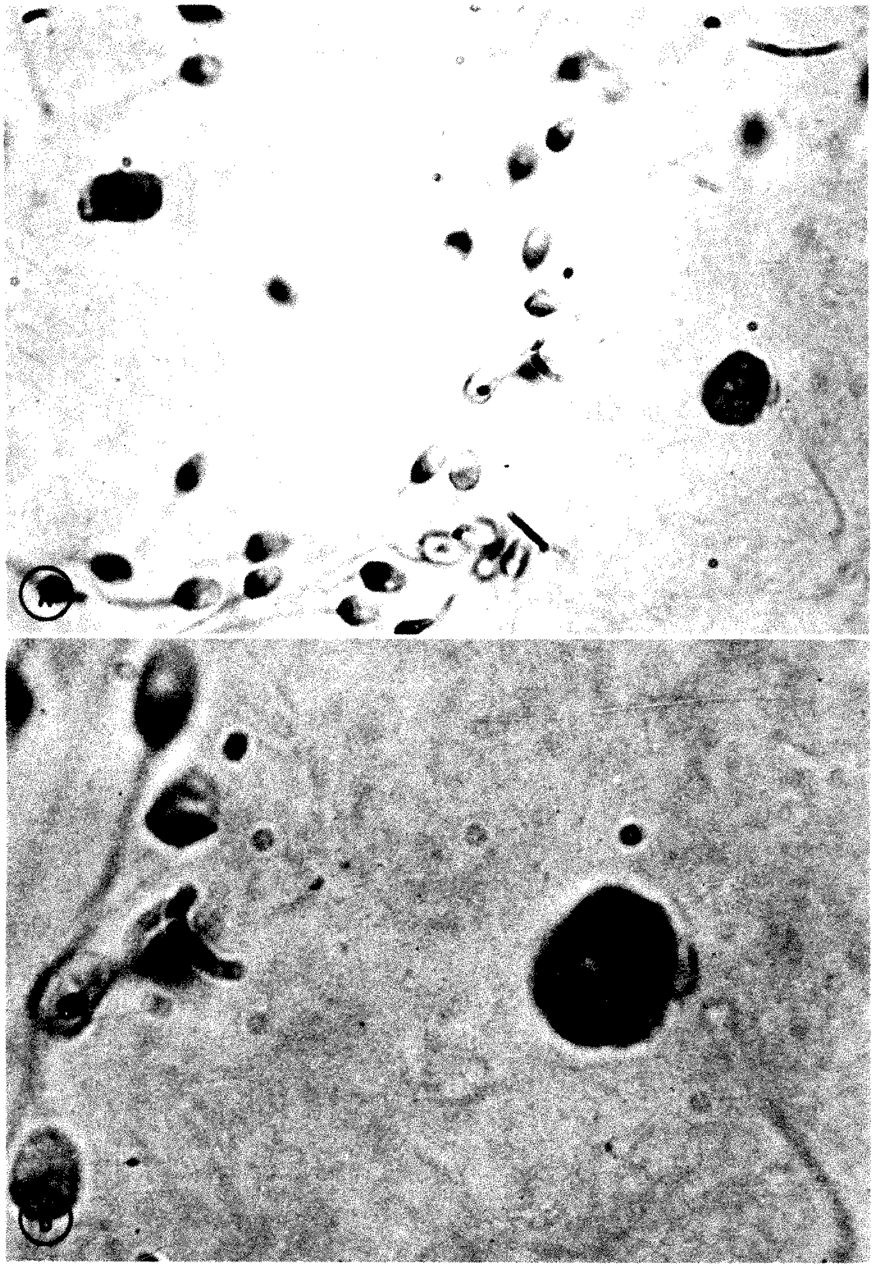

Fig. 2. Swollen human sperm head and corresponding sperm tail within the ooplasm of a penetrated hamster ovum with spermatozoa of normal size and shape that did not penetrate in the background *(A)*. (×3500.) The same human sperm within the penetrated ovum, magnified 7500 times *(B)*.

tolacmoid and examined microscopically for evidence of sperm incorporation. Ova are considered penetrated when a swollen sperm head or a male pronucleus and a corresponding sperm tail are found within the ooplasm. The penetration capacity of the Y-enriched sperm is tested against that of the nonpreselected ejaculate. Each preselected sample is also examined microscopically for the presence of F-bodies in the sperm heads by means of the quinacrine dihydrochloride staining method (Fig. 1).[1]

In the present experiment, ejaculates from 12 fertile donors were tested. The results, as presented in Table I, show that utilization of the single-column four-step four-layer albumin gradient technique provides (1) excellent Y-enriched sperm motility, (2) efficient separation of spermatozoa, yielding highly Y-enriched sperm fractions, and (3) high fertilizing capacity of these fractions as tested by the penetration capacity of the sperm into zona-free hamster ova, even after 18 hours of preincubation (Fig. 2).

In view of these satisfactory results, the clinical application of the described method, in an attempt to prevent sex-linked genetic diseases, is encouraged.

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Umbilical cord pH, P_{CO_2}, and bicarbonate following uncomplicated term vaginal deliveries

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Normal values for umbilical arterial and venous pH, P_{CO_2}, P_{O_2}, and bicarbonate must be known before these parameters can be used for assistance in clinical decisions. We evaluated the cord blood from 146 infants born after uncomplicated labor and vaginal deliveries at 37 to 42 weeks' gestation. All infants had a normal baseline fetal heart rate and normal beat-to-beat variability for at least 10 minutes preceding expulsion. The cord blood of infants born to women with pregnancy complications such as diabetes mellitus, preeclampsia, twins, meconium-stained amniotic fluid, or fetal growth retardation was not included. Mean umbilical arterial values ± 1 SD for the parameters studied were: pH, 7.28 ± 0.05; P_{CO_2}, 49.2 ± 8.4 mm Hg; P_{O_2}, 18.0 ± 6.2 mm Hg; bicarbonate, 22.3 ± 2.5 mEq/L. Umbilical venous values were: pH, 7.35 ± 0.05; P_{CO_2}, 38.2 ± 5.6 mm Hg; P_{O_2}, 29.2 ± 5.9 mm Hg; bicarbonate, 20.4 ± 4.1 mEq/L. (AM J OBSTET GYNECOL 1985;151:798-800.)

Key words: Fetal heart rate, cord blood gas values

Analysis of umbilical cord arterial and venous blood for acid-base and blood gas parameters can be of benefit in retrospectively assessing the intrapartum condition of the fetus and in evaluating obstetric techniques for the management of labor. Apgar scores can assist in the differentiation between normal and severely compromised neonates but may not be sensitive enough to allow delineation of less obvious neonatal morbidity.[1] Recent interest has focused, therefore, on umbilical cord blood pH as an index of intrapartum management and as a potential benchmark for neonatal outcome. Gilstrap and Hauth[2] correlated baseline fetal heart rate patterns during the last 10 minutes of the second stage of labor with umbilical cord acidemia. In addition, they compared the neonatal status at delivery to umbilical cord acid-base values and to the method of delivery.[3] Wible et al.[4] also used umbilical cord acid-base determinations in perinatal surveillance and management. They noted a correlation between neonatal acid-base status and intrapartum events and further attempted to differentiate between respiratory and metabolic acidosis. The increasing interest in the use of umbilical cord acid-base determinations to assess intrapartum events makes it important to define more precisely the normal values of these parameters. Prior attempts to do so[1, 4-11] were flawed by many potential complicating factors. Most importantly, no one has reported normal umbilical arterial or venous blood gas values for a sizable group of term patients with uncomplicated vaginal deliveries and a normal baseline fetal heart rate and normal beat-to-beat variability. Our purpose was, therefore, to define normal values of pH, P_{CO_2}, P_{O_2}, and bicarbonate in umbilical arterial and venous blood. Efforts were directed toward excluding many variables which could potentially adversely affect these values.

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Table I. Umbilical arterial and venous pH and blood gas determinations

	Arterial				*Venous*			
	Mean	*SD*	*Range*	*SEM*	*Mean*	*SD*	*Range*	*SEM*
pH	7.28	0.05	7.15-7.43	0.004	7.35	0.05	7.24-7.49	0.004
P_{CO_2} (mm Hg)	49.2	8.4	31.1-74.3	0.68	38.2	5.6	23.2-49.2	0.46
P_{O_2} (mm Hg)	18.0	6.2	3.8-33.8	0.50	29.2	5.9	15.4-48.2	0.48
Bicarbonate (mEq/L)	22.3	2.5	13.3-27.5	0.20	20.4	2.1	15.9-24.7	0.17

Material and methods

The present report is derived from data collected at Wilford Hall United States Air Force Medical Center from February 1, 1983, through May 31, 1983. During this period both umbilical arterial and venous cord blood samples were collected following virtually all vaginal deliveries.

Cord pH and gas determinations. A segment of umbilical cord was doubly clamped immediately after delivery of the infant. A 2 ml sample of blood was drawn into a plastic syringe that had been flushed with 1000 units/ml of heparin solution; a sample was collected from both the umbilical vein and the umbilical artery for each delivery. The blood gas determinations were then performed on an ABL-2 analyzer (Radiometer, Copenhagen, Denmark) within 5 minutes of collection.

Maternal factors. Values obtained from cord blood of infants with an estimated gestational age <37 or >42 completed weeks were not included in this report. Also excluded were infants born to women with diabetes, preeclampsia, multiple gestation, Rh disease, or other pregnancy complications. Delivery was spontaneous in 111 patients and by outlet forceps in 35.

Fetal factors. The cord blood of infants with criteria suggestive of growth retardation, with meconium staining of the amniotic fluid, or with identifiable fetal anomalies was excluded. Infants with significant fetal heart rate abnormalities during the first stage of labor, such as late decelerations, severe variable decelerations, or any persistent nonperiodic patterns of bradycardia or tachycardia, were also excluded from analysis. All neonates weighed >2500 gm. The above exclusions resulted in the analysis of gas and acid-base parameters in the cord blood of 111 infants born spontaneously and in 35 infants delivered by outlet forceps.

Fetal heart rate monitoring. All infants evaluated had at least a 10-minute segment of recorded fetal heart rate via a scalp electrode during the end of the second stage of labor but prior to expulsion. The fetal heart rate tracings were reviewed independently by two of the authors (J. C. H. and L. C. G.) without knowledge of the umbilical cord acid-base values. All patients with periodic fetal heart rate abnormalities, baseline tachycardia or bradycardia, and the absence of beat-to-beat variability were excluded from analysis. Those cases in which there was disagreement concerning the interpretation of the tracing were excluded.

Table II. Normal values for umbilical arterial and venous pH and blood gases in other reports

Source	*No. of patients*	*Mean umbilical arterial pH*	*SD*
Sykes et al.[1]	899	7.20	0.08
Wible et al.[4]	29	7.24	–
Koch and Wendel[5]	27	7.24	0.06
Hill et al.,[6] Longo et al.,[7] Power et al.[8]	*	7.35	–
Clark et al.[9]	39	7.22	0.06
Beard[10]	18	7.20	0.08
Mondanlou et al.[11]	38	7.25	–

*See text.

Results

The means, standard deviations, and ranges for pH, P_{CO_2}, P_{O_2}, and bicarbonate in the umbilical arterial and venous cord blood of 146 neonates are listed in Table I.

Comment

Assessment of neonatal status by umbilical cord blood acid-base determinations represents an alternative to the Apgar score. Clearly, it would be difficult to infer that perinatal depression resulted from intrapartum events if umbilical cord blood acid-base values were normal, regardless of the Apgar scores. Cord blood analysis is convenient and the values are determined objectively, whereas the Apgar score is more subjective. The number of variables which could potentially influence umbilical cord pH and blood gas values seems almost limitless. However, the factor believed to be most predictive of fetal well-being is a normal baseline fetal heart rate. The addition of a normal beat-to-beat fetal heart rate variability provides added evidence of fetal "normalcy,"[12] although not all reports would include the presence of beat-to-beat variability.[13]

That a fetus can be consistently identified as normal by means of continuous electronic fetal heart rate monitoring is generally accepted.[14] Gilstrap and Hauth[2] also reported that a normal baseline fetal heart rate and the presence of beat-to-beat variability were 97% correlative with no neonatal acidemia (defined as a cord blood pH of <7.20).[2] Therefore, as the key criterion for defining normal umbilical arterial and venous pH and blood gases for the present study, we selected only term gestations for which a fetal heart rate tracing was demonstrative of a normal baseline and beat-to-beat variability for the 10 minutes preceding expulsion. Moreover, infants born to women with pregnancy complications were excluded since Modanlou et al.[11] reported significant differences in umbilical cord blood acid-base parameters when high-risk pregnancies were compared to normal pregnancies. We did not, however, simultaneously measure pH in the maternal compartment as suggested by Miller et al.[15] but are aware that some of the pH values obtained could be artifactually elevated by maternal hyperventilation. However, the 20 women in his study were all hyperventilated intentionally. We are not aware of a report that defines normal newborn umbilical artery and venous acid-base values in relation to spontaneous maternal respiratory patterns in the second stage of labor. In addition, we did not control for duration of the first or second stage of labor or type of anesthesia and analgesia used for labor and delivery. Wood et al.,[16] however, found no significant acid-base changes related to the total duration of the second stage of labor unless specific delays in delivery occurred. These variables were addressed in that our selection criteria included a normal baseline fetal heart rate and beat-to-beat variability just prior to expulsion. In practice, these fetal heart rate criteria provide obstetricians assurance of fetal well-being and normalcy and most importantly provide guidance as to when second-stage operative intervention is indicated for potential fetal compromise.[2]

Several other reports have addressed the subject of normal values for umbilical arterial and venous pH and blood gases.[1, 4-11] The number of patients in each study, the mean umbilical artery pH, and a standard deviation, if reported, are listed in Table II. In these reports variables used to identify a "normal" population variously included length of gestation, labor complications, pregnancy complications, route of delivery, application of continuous fetal heart rate monitoring, and fetal scalp pH monitoring.[4, 5, 9-11] Some authors[6-8] predicted a normal mean umbilical arterial pH in human newborn infants by extrapolating animal data. Sykes et al.[1] determined the mean umbilical arterial pH and standard deviation for a large number of consecutive deliveries although many of the aforementioned variables were not controlled. Most serious of these omissions was that continuous fetal heart rate monitoring was used in less than half of the patients in their series.

We anticipate that increasing attention will be focused on the acid-base status of the newborn infant. This biochemical status will then be retrospectively correlated with obstetric management and anterospectively correlated with short- and long-term neonatal outcome. Normal mean values and standard deviations for umbilical artery and vein pH, P_{CO_2}, P_{O_2}, and bicarbonate have been provided for 146 selected vaginal deliveries.

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Prolactin production by explants of normal, luteal phase defective, and corrected luteal phase defective late secretory endometrium

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The production of prolactin by explants of late secretory endometrium has been correlated with the extent of decidual differentiation. This correlation is strengthened by the observation that luteal phase defective endometrium produces less prolactin than normal control endometrium in a 24-hour in vitro culture system. In the present study the prolactin production by explants of normal, luteal phase defective, progesterone-corrected luteal phase defective, and clomiphene– or follicle-stimulating hormone/luteinizing hormone–corrected luteal phase defective late secretory endometrium was measured over 96 hours at 24-hour intervals. Progesterone in physiologic concentrations was added to the culture medium to maintain tissue integrity and prolactin synthesis. The prolactin production of normal late secretory endometrium rose over 96 hours under progesterone stimulation. The luteal phase defective endometrium produced significantly less prolactin under the same conditions. Histologically proven corrected luteal phase defective endometrium, regardless of treatment method, produced prolactin not different from the normal controls of the same dates. From these results it is concluded that histologic correction of luteal phase defective endometrium is associated with a corresponding biochemical correction with use of prolactin as a metabolic marker. The findings also strongly support timed endometrial biopsy as the method of diagnosis and evaluation of treatment of luteal phase defect. (AM J OBSTET GYNECOL 1985;151:801-4.)

Key words: Endometrial prolactin, luteal phase defect

The luteal phase defect as a cause of infertility and early pregnancy wastage has been recognized since 1949.[1] It is reported to affect 8.1% of infertility patients in a infertility referral practice.[2] Numerous studies regarding its clinical significance and methods of diagnosis have appeared in the literature.[3] A commonly used method of diagnosis is the endometrial biopsy. The timed biopsy documents secretory maturation and decidualization of luteal phase endometrium by histologic criteria established by Noyes et al.[4]

The decidualization of normal endometrium is not only associated with characteristic histologic patterns but is also accompanied by de novo synthesis and release of prolactin by the endometrium.[5] The prolactin production is detectable on day 23 of an ideal menstrual cycle and increases with increasing decidualization. On the other hand the in vitro production of prolactin by luteal phase defective late secretory endometrium over 24 hours is less than in normal control endometrium of the same ideal menstrual dates.[6] Furthermore, progesterone is required in the incubation medium to maintain morphologically intact endometrial explants and prolactin production in vitro.[7]

The present study was undertaken to further correlate the histologic decidualization with late secretory prolactin production by comparing 24-hour prolactin production of normal, luteal phase defective, and corrected luteal phase defective endometrial explants over 96 hours. The study also investigated the correlation of histologic and biochemical correction of luteal phase defect and the response in vitro to progesterone in luteal phase defective endometrium.

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Material and methods

After appropriate consent and approval of the Human Research Committee, endometrium was obtained by endometrial biopsy as part of the evaluation of infertility in patients with normal menstrual cycles by history and basal body temperature. The onset of menses following biopsy was carefully recorded. A portion of each biopsy was preserved for histologic evaluation. The remaining tissue was minced to 2 × 2 mm pieces and divided into aliquots by wet weight of 50 ± 2 mg. This tissue was cultured in triplicate in Dulbecco's modified Eagle's medium containing 0.1% gelatin, 10 mmol/L HEPES (final pH, 7.4 to 7.6), 2.5 IU/ml of nystatin, 0.1 mg/ml of Garamycin, 145 U/ml of penicillin, 145 U/ml of streptomycin and 20 ng/ml of progesterone. Tissue was placed in 25 ml Erlenmeyer flasks containing 2 ml of medium and oxygenated with 95% oxygen and 5% carbon dioxide. It was then incubated in a 37° C water shaker bath. Medium was sampled and changed at 24, 48, 72, and 96 hours. After medium aspiration at 96 hours, tissue was fixed in formalin for histologic evaluation. Culture medium removed was frozen at −20° C for later prolactin assay. Prolactin concentration in all samples was estimated by radioimmunoassay as previously described by Sinha et al.[8] Radioactive human prolactin was purchased from New England Nuclear, North Billerica, Massachusetts. Prolactin antibody was provided by the National Institute of Arthritis and Metabolic Diseases (No. AFP-C11580), Bethesda, Maryland. The amount of prolactin produced over 24 hours of incubation was calculated and then normalized to nanograms of prolactin per 100 mg of tissue wet weight.

Dating of the endometrial biopsies. Samples of endometrium obtained from 25 patients were included in this study. Biopsies were performed on the twelfth day after ovulation by basal body temperature chart. Twenty-four patients were ovulatory by basal body temperature and one patient was treated with clomiphene successfully for anovulation. Eight patients were receiving treatment for previously diagnosed luteal phase defect; five with progesterone suppositories, 25 mg twice daily starting on luteal day 3 based on temperature nadir; one with clomiphene citrate, 50 mg on days 5 to 9 plus dexamethasone, 0.5 mg daily at bedtime; two with follicle-stimulating hormone/luteinizing hormone, 1 ampule on days 2 to 6.

Each tissue was dated histologically by the criteria of Noyes et al[1] and then compared with the subsequent onset of menses in the biopsy cycle. Histologic findings were read without a prior knowledge of the date of onset of menses or prolactin synthesis. When histologic findings agreed within 2 days of the date determined by menses, the tissue was considered normal. When the histologic date lagged the expected date by ≥2 days, the biopsy was diagnosed as out of phase. A repeat biopsy of the same findings confirmed luteal phase defects in all abnormal biopsies.

Since prolactin production in the late luteal phase increases significantly every 2 days after ideal cycle day 23,[6] prolactin production by normal endometrium of histologic days 25 and 26 was grouped together for the purpose of statistical evaluation. Prolactin values of luteal phase defective tissues were grouped together regardless of actual histologic dating. After 96 hours of incubation, endometrial tissues consisted of a central area of necrosis surrounded by a healthier layer of decidual cells and secretory-appearing glands. Tissues showing generalized necrosis were excluded from this study. Seven normal tissues were thus excluded. A likelihood ratio—χ^2 analysis was applied for statistical evaluation with level of significance defined as $p < 0.05$.

Results

Samples of endometrium were found to be histologically normal relative to subsequent menses in 21 patients and abnormal in 4 patients. These samples were classified as normal endometrium (group A), luteal phase defective endometrium (group B), progesterone-corrected luteal phase defective endometrium (group C), and clomiphene– or follicle-stimulating hormone/luteinizing hormone–corrected luteal phase defective endometrium (group D). Group A represents 13 patients with apparently normal, nonmedicated cycles. Group B includes one patient receiving clomiphene for anovulation, 1 patient receiving clomiphene for previously diagnosed luteal phase defect who was later found to have hyperprolactinemia, and two nonmedicated patients with luteal phase defects. Group C represents five progesterone-treated patients with histologic correction of luteal phase defect. Group D includes one patient receiving clomiphene and dexamethasone whose luteal phase defect did not correct on progesterone treatment, and two patients receiving follicle-stimulating hormone/luteinizing hormone whose luteal phase defect did not correct on receiving progesterone and clomiphene. The management protocol for luteal phase defects was described previously by Daly et al.[9]

Normal endometria incubated with progesterone showed significant increase of prolactin production from 24 to 48 and from 48 to 72 hours ($p < 0.01$) (Fig. 1). No further increase was detected after 72 hours. Luteal phase defective endometria incubated with progesterone showed a significant increase of prolactin production as well from 24 to 48 hours ($p < 0.05$) (Fig. 1), but no further significant change occurred thereafter. Groups C and D both demonstrated no significant increase from 24 to 48 hours ($p = 0.08$ and 0.06, re-

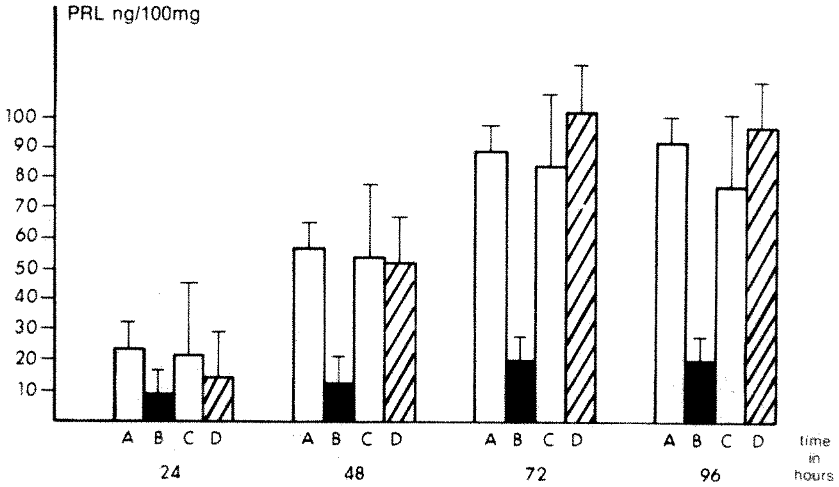

Fig. 1. Prolactin production by late secretory endometrial explants (mean ± SEM). Group *A*, normal endometrium; group *B*, luteal phase defective endometrium; group *C*, progesterone-corrected luteal phase defective endometrium; group *D*, clomiphene–or follicle-stimulating hormone/luteinizing hormone–corrected luteal phase defective endometrium. I. Prolactin production over time: group *A*—$p(24\text{-}48) < 0.01$, $p(48\text{-}72) < 0.01$, $p(72\text{-}96) = \text{NS}$; group *B*—$p(24\text{-}48) < 0.05$, $p(48\text{-}72) = 0.07$, $p(72\text{-}96) = \text{NS}$; group *C*—$p(24\text{-}48) = 0.08$, $p(48\text{-}72) < 0.05$, $p(72\text{-}96) = \text{NS}$; group *D*—$p(24\text{-}48) = 0.06$, $p(48\text{-}72) < 0.05$, $p(72\text{-}96) = \text{NS}$. II. Between-group comparisons of prolactin level at 24, 48, 72, and 96 hours: 24 hours—$p(AB) = 0.07$, $p(AC) = \text{NS}$, $p(AD) = \text{NS}$; 48 hours—$p(AB) < 0.01$, $p(AC) = \text{NS}$, $p(AD) = \text{NS}$; 72 hours—$p(AB) < 0.01$, $p(AC) = \text{NS}$, $p(AD) = \text{NS}$; 96 hours—$p(AB) < 0.01$, $p(AC) = \text{NS}$, $p(AD) = \text{NS}$.

spectively) but a significant increase from 48 to 72 hours ($p < 0.05$). No further increase was detected after 72 hours (Fig. 1).

When prolactin production between groups was compared at different times in culture, no difference was found between groups A, B, C, and D at 24 hours. A significant difference between groups A and B was observed at 48 hours and thereafter. No difference was detected between groups A and C and between groups A and D at any time.

Comment

Human decidua has been demonstrated to produce prolactin in organ culture[10] and is considered to be the main source of prolactin present in amniotic fluid.[11] The source of prolactin in human decidua has recently been isolated to be decidual cells.[12] The amount of immunoreactive prolactin produced by late secretory endometrium is a function of the extent of decidual differentiation.[6] This function was further documented when luteal phase defective endometrium was found to produce less prolactin than normal control endometrium of the same menstrual date.[6]

To maintain decidualization and prolactin production of late luteal phase endometrial explants, progesterone was found to be necessary. Progesterone alone has previously been shown capable of inducing decidualization with attendant prolactin production in culture of proliferative endometrium.[13] The hormonal actions of progesterone on endometrium can be correlated with the induction of endometrial progesterone receptors that increase to a peak level in the late follicular phase and gradually decrease to a nadir prior to menses.[14, 15]

Based on the above considerations, progesterone in physiologic concentration was added to the culture medium to support explant integrity and prolactin production. Prolactin synthesis and release by normal endometrium confirmed findings reported previously.[7] Compared to normal control endometrium, luteal phase defective endometrium not only produced less prolactin but also demonstrated a lack of response to progesterone supplementation in culture. This may be explained by the observation that normal late secretory phase endometrium contains only a small amount of progesterone receptors, both cytoplasmic and nuclear,[14] and even fewer receptors have been observed in luteal phase defective tissue.[16] This may explain the lack of clinical efficacy of progesterone treatment unless started early in the luteal phase.

No difference in prolactin production or histology at 96 hours was found between endometrial explants from corrected luteal phase defective tissue and normal control tissue. The method of treatment did not seem to matter. Compared with normal control tissue, the histology of luteal phase defective tissue at 96 hours showed smaller stromal cells with ground glass–appearing cytoplasm. The progesterone-corrected luteal phase defective tissue must be assumed to have had adequate endometrial progesterone receptors in the early secretory phase, allowing induction of mature decidualization. The induction of progesterone receptors, on the other hand, is dependent on estradiol synthesis during folliculogenesis.[15, 16] Thus the endometrial cycle corresponds to the ovarian cycle so that inadequate folliculogenesis results in inadequate induction of endometrial progesterone receptors. By improving folliculogenesis, clomiphene- and follicle-stimulating hormone/luteinizing hormone-corrected luteal phase defective patients who failed to respond to progesterone previously were able to achieve mature decidualization. If histologic maturity cannot be achieved, the prognosis for viable pregnancy is extremely poor.[9]

In conclusion, histologic correction of luteal phase defects is associated with corresponding biologic correction when prolactin is used as the biochemical marker. This correlation seems to be independent of the treatment method.

In view of the recent clinical study of endometrial biopsy being predictive of therapeutic outcome in luteal phase defective patients,[9] this study further establishes timed endometrial biopsy as the preferable method for diagnosis and evaluation of treatment of the luteal phase defect.

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Gap junctions and myometrial steroid hormone receptors in pregnant and postpartum rats: A possible cellular basis for the progesterone withdrawal hypothesis

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Myometrial gap junctions, levels of cytosol and nuclear estradiol and progesterone receptors, and serum estradiol and progesterone levels were determined simultaneously in pregnant and postpartum rats. Between days 15 to 20 after conception, levels of progesterone nuclear receptors decreased (776.8 ± 88.5 versus 241.1 ± 56.5 fmol/mg of DNA, $p < 0.05$). The mean serum progesterone also fell by about 50% during this period, but variation in individual levels between days 15 to 20 did not allow for this group to achieve statistical significance until day 21. As the progesterone nuclear receptors decreased, estradiol remained stable, but estradiol nuclear receptors increased (day 21 versus 22: 1710.6 ± 61.1 versus 3254.8 ± 203.8 fmol/mg of DNA, $p < 0.05$), preceding the increase in gap junctions observed at parturition (day 21 versus 22: 2.0 ± 0.2 versus 8.0 ± 3.2 [per 1000 μm plasma membrane], $p < 0.05$). Gap junctions fell to prepartum levels by 12 hours, when progesterone nuclear receptors were markedly increasing (6 versus 12 hours post partum: 637.7 ± 324.8 versus 1509.6 ± 283.2 fmol/mg of DNA, $p < 0.05$). Relationships between gap junctions and cellular estradiol and progesterone nuclear receptors are clearer than could be forecast by circulating hormone measurements alone, and may offer a cellular basis for the role of progesterone in controlling labor. (Am J Obstet Gynecol 1985;151:805-12.)

Key words: Gap junction, hormone receptors, progesterone withdrawal

The preparation of the uterus for labor is complex, tightly orchestrated, and steroid hormone dependent.[1] It has been more than 20 years since Csapo et al.[2] postulated that progesterone withdrawal is a primary event controlling the onset of labor. The theory was based upon changes in the circulating/extracellular progesterone and later enlarged to include changes in the estrogen/progesterone ratio. Perhaps because the presence of intracellular steroid hormone receptors was reported some years after Csapo's original formulation of the mechanism of labor, this aspect has not received much attention.

Recent work by Puri and Garfield[3] and Garfield et al.[4] indicating that gap junction formation, a steroid-dependent phenomenon, may control the onset of concerted uterine contractility, gave us an opportunity to assess the relative roles of circulating hormones and intracellular receptor mechanisms in relation to the "progesterone withdrawal" hypothesis. We performed the studies described below and found a closer relationship between the receptor kinetics and gap junctions than between serum steroids and gap junctions.

Material and methods

Animals. Timed pregnant Sprague-Dawley rats (CD strain) were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and maintained individually under a 12-hour light: 12-hour dark cycle with water and Purina laboratory chow ad libitum. Groups of three to five rats were decapitated at 10:00 AM on days 15, 20, 21, and 22 after conception, during delivery (all rats began delivery in the morning of day 22 and were killed when 1 to 5 fetuses had been delivered), and post partum (3, 6, and 12 hours after delivery), and trunk blood serum was obtained for steroid radioimmunoassay. The uteri were quickly removed, freed of fat and mesenteries, and processed as described below.

Thin-section electron microscopy. Longitudinal strips of antimesenteric uterine tissue were prepared by a modification of the method of Garfield et al.[4] One third of the uterine horn (not including placental implantation sites) was stretched to about 1.5 times the

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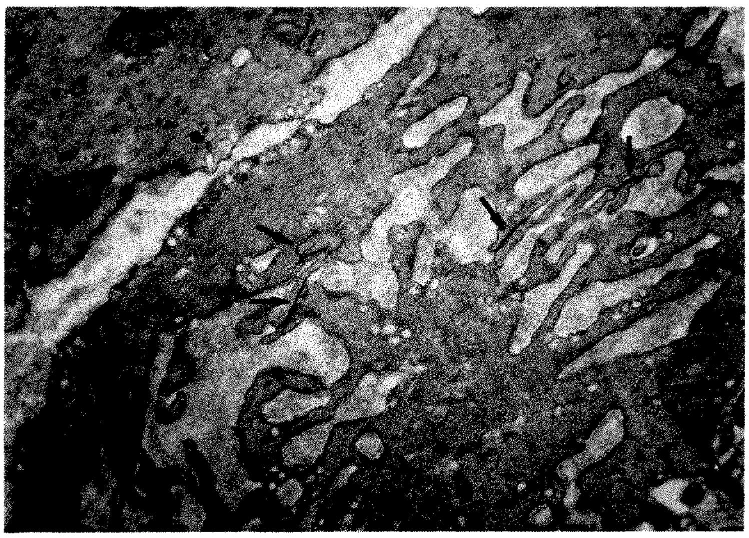

Fig. 1. Multiple gap junctions *(arrows)* between myometrial cells from the uterus of a 22-day pregnant rat. (final magnification, ×20,000.)

contracted excised length and was pinned out on a plastic strip in medium 199 (Grand Island Biochemical Company). The stretched uterine tissue strips were fixed by immersion in 2.0% glutaraldehyde (+4.5% sucrose, 1 mmol/L of calcium chloride in 75 mmol/L of sodium cacodylate buffer, pH 7.4) for about 1.5 hours at room temperature. Thereafter the tissue was cut into small pieces (about 0.1 × 0.1 mm) in the fixative and left for an additional 30 minutes.

Following the initial glutaraldehyde fixation, all tissues were washed in cacodylate buffer (100 mmol/L of sodium cacodylate buffer, pH 7.4, plus 6% sucrose plus 1.25 mmol/L of calcium chloride) overnight at 0° to 4° C and postfixed in 2% osmium tetroxide (in 50 mmol/L of cacodylate buffer, pH 7.4) for 90 minutes. All tissues were stained en bloc with saturated uranyl acetate for 40 minutes, dehydrated in graded ethanols, and embedded in Spurr resin. The tissues were oriented in molds in order to separately cut the outer longitudinal and inner-circular muscle layers in cross section. Sections of muscle were stained for 15 minutes with lead citrate and examined in a Hitachi HU-12 electron microscope at 75 kV. As part of the methodologic development the morphology of specimens following immersion fixation was compared with specimens obtained following intracardiac perfusion fixation. Since there was no difference in terms of the quality of ultrastructural preservation, the immersion fixation method was used for the studies reported below.

Morphometry. Ten to 15 random nonoverlapping photographs from one grid of each cross section of a muscle layer were examined at ×10,000 magnification. Prints were made by enlarging the negatives 3.3 times (×33,000) on 20 × 25 cm paper. A Bioquant II (Kramer Scientific Corporation) was used to estimate the length of smooth muscle cell membrane observed and to trace gap junctions in the photographs. The gap junctions were identified by their five- to seven-lined structure (Figs. 1 and 2). The length of each gap junction was directly calculated from the tracings. To estimate the length of smooth muscle cell membrane in the photograph, a transparent grid (1.0 × 1.0 cm, 24 × 18 lines) was applied over the photograph. The total length of membrane in the photographs was determined by counting intersections of the grid with the plasma membrane and applying the formula of Weibel[5]: $L = d \times a/2 \times 1/2$, where L = length of membrane, d = correction factor for magnification (1/33), and a = number of intersections minus 1.

Steroid hormone radioimmunoassay. Trunk blood serum was assayed for unconjugated estradiol 17-β after ethyl acetate/hexane extraction by means of a ^{125}I-estradiol radioimmunoassay kit and for progesterone by means of a ^{125}I-progesterone radioimmunoassay kit on unextracted serum samples. Both kits were obtained from Radioassay Systems Laboratories, Inc. (Carson, California). The specificity for estradiol was high (100%), with cross-binding for estriol, 6.5%; estradiol 17α, 5.2%; and estrone, 0.55%. The progesterone assay cross-reacted with 20α dihydroprogesterone, 6.25%; desoxycorticosterone, 3.2%; 17α-hydroxyprogesterone, 0.15%; and cortisol, <0.1%. All samples were assayed in duplicate in the same assay. The intra-assay variance was less than 8%.

Steroid hormone receptor assay

Chemicals. [2,4,6,7-^{3}H]Estradiol (115 Ci/mmol), [17-α-methyl-^{3}H]R5020 (87 Ci/mmol), and nonradioactive R5020 were obtained from New England Nuclear (Boston, Massachusetts). Radioligands were checked for purity (>95%) by LH-20 column chromatography. Dithiothreitol and diethylstilbestrol were obtained from Sigma Chemical Company (St. Louis). All reagents were of the highest purity available.

Preparation of cytosol and nuclear extract. After removing small strips from the uterine horn for electron microscopy, the conceptuses were removed, endometrium was scraped off, and tissue was washed with ice-cold saline solution to remove blood and amniotic fluid. Thereafter, the remaining right and left horns were placed in 10% dimethyl sulfoxide (Mallinckrodt, St. Louis) to minimize tissue damage caused by freezing,[6] frozen on dry ice, and stored at −70°. For receptor assay, the horns were thawed on ice, washed in ice-cold saline solution to remove the dimethyl sulfoxide, minced finely on a chilled Petri dish, and then homogenized in a glass-glass Potter homogenizer in 10 mmol/L of Tris hydrochloric acid buffer, pH 7.4, with 1.5 mmol/L of sodium ethylenediaminetetra-acetate, 1 mmol/L of dithiothreitol and 10% (v/v) glycerol (30 ml/gm of tissue; TEGD buffer). The homogenates were centrifuged at 3,000 × g for 10 minutes at 4° C. The supernatant was decanted and further centrifuged at 105,000 × g for 45 minutes to separate cytosol. The cytosol was then passed through a Sephadex LH-20 column (0.7 × 2.0 cm) equilibrated with TEGD buffer to remove endogenous steroids before assay.

For myometrial cell nuclear preparations, the myofibrillar pellets from the initial centrifugation were immediately washed with 3 × 10 ml of washing buffer (0.32 mol/L of sucrose, 1 mmol/L of monobasic potassium phosphate, 3 mmol/L of magnesium chloride, 1 mmol/L of dithiothreitol, 10% v/v glycerol, and 1 mg/ml of bacitracin, pH 7.3), and the pellets resuspended in 0.8 ml of washing buffer, to which an additional 10 ml of resuspending buffer (same as washing buffer, with the addition of 2.4 mol/L of sucrose) were added with vigorous vortexing. Tubes were left on ice for 5 minutes and centrifuged at 20,000 × g for 30 minutes in a Sorvall RC2-B centrifuge, with use of an HB-4 swinging bucket rotor. The supernatant was discarded, and the tube wall was cleaned and dried. The purified nuclear pellets were then resuspended in 3 ml of 0.5 mol/L of potassium chloride (in TEGD buffer) and extracted for 30 minutes. The extracted nuclear receptors were separated from the pellets by centrifugation at 20,000 × g for 15 minutes. DNA content of the pellets was determined by the method of Burton,[7] and cytosol protein was measured as described by Bradford.[8]

Steroid receptor assay. Aliquots (200 μl) of LH-20 stripped cytosol or nuclear receptor extract were incubated with either [2,4,6,7-^{3}H]estradiol or [17α methyl-^{3}H]R5020 for assay of estradiol and progesterone receptors, respectively. The final concentration of radioligand in each case was 5 nmol/L. The nonspecific binding was determined by the parallel incubation of the sample in the presence of either 50 molar excess of diethylstilbestrol (for estradiol receptors) and 100 molar excess of R5020 (for progesterone receptors). The final incubate volume was adjusted with buffer to 300 μl. Incubations were carried out for either 4 hours at 0° to 4° C (for progesterone receptors) or overnight at 0° to 4° C followed by 4.5 hours' incubation at 25° C (for exchange assay of total estradiol receptors). After incubation, aliquots (100 μl) were withdrawn from the incubate and subjected to Sephadex LH-20 minicolumn chromatography as reported previously.[8] As a preliminary experiment, both unoccupied and occupied estradiol nuclear receptors were measured during the indicated time of pregnancy. Since more than 90% of the fraction of total estradiol nuclear receptors was the occupied form and since the ratio of unoccupied to occupied remained constant, the assays of estradiol nuclear receptors were performed at the exchange (i.e., 25° C) condition to determine total estradiol nuclear receptors. Labeled receptors in the void volume were collected, and the radioactivity was counted in 5 ml of Betafluor (National Diagnostics) in a Beckman LS 8000 liquid scintillation counter at an average counting efficiency of 50%.

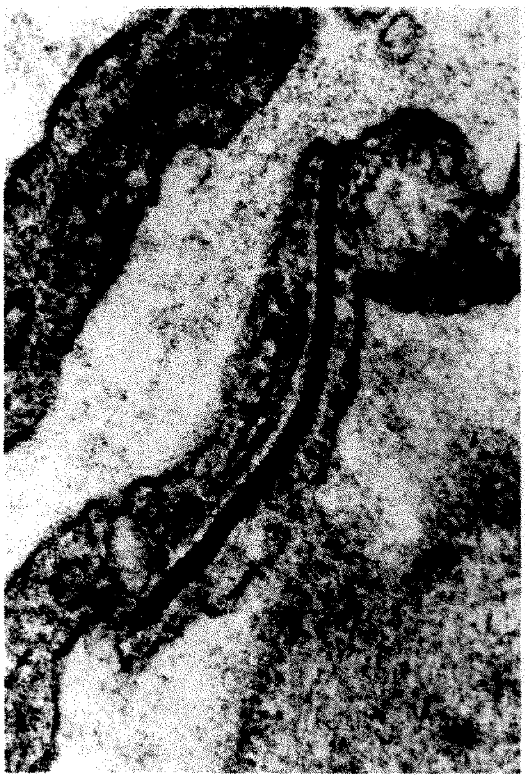

Fig. 2. High-power magnification (final magnification, ×300,000) of a gap junction observed during labor. Characteristic seven layers, four dark and three light, are identified.

Table I. The number of horns, total number of gap junctions, and the length and the number of gap junctions per unit length of plasma membrane

Day	*No. of horns studied*	*No. of gap junctions*	*Length (μm)**	*No. of gap junctions per 1000 μm of plasma membrane**
15	6	4 (2/6)†	0.16 ± 0.02	4.2 ± 0.0
20	10	4 (4/6)	0.18 ± 0.02	3.6 ± 0.3
21	10	6 (4/10)	0.13 ± 0.05	2.0 ± 0.4
22				
−6 hr	10	26 (10/10)‡	0.25 ± 0.02‡	8.0 ± 3.2‡
0	6	39 (6/6)‡	0.29 ± 0.02‡	17.9 ± 1.5‡
+3 hr	6	43 (6/6)‡	0.27 ± 0.02‡	26.0 ± 9.5‡
+6 hr	6	49 (6/6)‡	0.25 ± 0.03‡	27.8 ± 4.2‡
+12 hr	6	7 (4/6)	0.18 ± 0.02	5.4 ± 1.0

*Mean ± SEM.

†In parentheses: horns with gap junctions/total horns.

‡Significantly higher than prepartum or postpartum levels ($p<0.05$, by Duncan's multiple range test).

Statistical comparisons. Results were analyzed by means of analysis of variance, the unpaired Student's *t* test and Duncan's multiple range test.

Results

Gap junctions in myometrial tissues from non-pregnant and postpartum rats. The gap junctions were present on apposition points where cytoplasmic protrusions from one cell to the soma of another met or where protrusions from two cells met (Figs. 1 and 2). Since there was excellent concordance between the number of gap junctions in outer longitudinal and inner circular muscle layers of myometrial tissues of late pregnant rats and since any differences were not statistically significant, the data from the two layers were combined for presentation and statistical comparisons. As shown in Table I, only a few gap junctions were observed in rat myometria on days 15, 20, and 21 after conception. No statistically significant change occurred until day 22 after conception (about 6 hours before delivery), when the number and size of gap junctions in both muscle layers abruptly began to increase. The maximum number and size of gap junctions were observed from 6 hours before to 6 hours after delivery. Both parameters declined rapidly thereafter: only a few gap junctions were found in the myometrial tissue of rats killed 12 hours after delivery (Fig. 3).

Similarly, the greatest average measured length of gap junctions in the myometrium was found during the 6 hours preceding and following labor and was significantly longer than that of days 15 to 21 after conception or 12-hour-postpartum animals (Duncan's test; $p < 0.05$) (Table I).

Serum steroid concentrations. Circulating estradiol and progesterone levels are shown in Fig. 3. Although the mean estradiol levels increased about 25% as delivery approached, there was considerable individual variation. Perhaps because of this individual variation, we could not detect a distinct peak of serum estradiol around the time of labor, as reported by others.[2] On the other hand, mean serum progesterone showed a steady fall between days 15 and 20; this was followed by a more rapid drop on day 22. A small but distinct peak of progesterone was observed at 12 hours after delivery, following postpartum ovulation.

Estrogen and progestin receptor concentrations. During the 24 hours immediately preceding delivery, there was a marked increase in both cytosol and nuclear total estradiol receptors. This increase was more apparent in the nuclear fraction, which more closely matched the serum estradiol rise during this period. The maximum concentration of estradiol nuclear receptors was observed 6 hours before the first delivery of a conceptus (day 21 after conception versus day 22 after conception: 1710.6 ± 61.1 versus 3254.8 ± 203.8 fmol/mg of DNA, mean ± SEM). In the first 3 hours after delivery, estradiol receptors in both the cytosol and the nuclear fraction declined with estradiol nuclear receptor changes preceding estradiol cytosol receptors. Levels of both estradiol cytosol receptors and nuclear receptors continued to decrease for the next 9 hours, reaching their lowest averages at the 12-hour postpartum point when the levels of progesterone nuclear receptors reached the highest average seen through the entire study. This was also the time that the gap junctions finally fell to prepartum levels (Fig. 3).

Mean progesterone nuclear receptors reached a nadir by days 20 and 21 after conception. On day 22 after conception (about 6 hours before delivery of the first pup), levels of both progesterone cytosol receptors and nuclear receptors in the myometrial tissue may have begun to increase but less rapidly than estradiol receptors. Subsequently, levels of both nuclear and cytosol progesterone receptors may have begun to rise slightly during the period 6 hours before and 3 hours following delivery compared to day 21 after conception ($p > 0.1$). Interestingly, this was the point of the lowest circulating progesterone level, and when the average serum estradiol level was at its highest point in the peripartum rise. The concentrations of progesterone nuclear receptors increased significantly 6 to 12 hours after parturition when levels of progesterone cytosol receptors, estradiol cytosol receptors, and estradiol nuclear receptors were all decreasing in the face of a rising serum progesterone level (Fig. 3).

Correlation between gap junction formation and concentration of steroid hormone receptors in myometria from pregnant and postpartum rats. The number and size of myometrial gap junctions increased abruptly on day 22 after conception, which corresponded in time with increased estradiol nuclear receptors and 24 hours of low progesterone nuclear receptors in the tissue. The maximum gap junctions were observed between 3 and 6 hours after delivery had begun, following by about 6 to 12 hours the curve which led to maximum concentrations of estradiol nuclear receptors in the same tissue and 48 hours after progesterone cytosol receptors reached their minimum levels. During the postpartum period the gap junctions diminished as did levels of estradiol nuclear receptors, while levels of progesterone nuclear receptors rose (Fig. 3).

Comment

The approach of labor is marked by changes in the estradiol and progesterone ratio which are accompanied by marked changes in the cervix and myometrium.[1] Among these prelabor effects, increased numbers and lengths of gap junctions between myometrial cells during or immediately prior to parturition have been reported in rats, mice, guinea pigs, sheep, and humans.[1-3, 9] However, little is known about the mechanism of formation of such structures. In view of the rapid increase of gap junctions before and during parturition Puri and Garfield[3] and Garfield et al.[4] have proposed that the formation of gap junctions between uterine muscle cells contributes to the initiation of labor by providing low resistance contacts between cells for coordinated, synchronized uterine contraction. They also hypothesized that the increasing ratio of tissue estradiol to progesterone following plasma progesterone withdrawal at term may lead to the increased formation of gap junctions.[3] However, circulating steroid levels are difficult to interpret because of high and changing levels of circulating and tissue α-fetoprotein—an estradiol binder present during rat pregnancy,[10] and tissue levels must be directly measured because of the extensive metabolism that may occur in the uterus itself.[11] In light of these facts, we decided to examine the system by studying estradiol and progesterone receptor kinetics, since they should present an integrated picture of the effects of steroid hormone interactions, which might only be partially controlled by circulating hormones and tissue metabolism.

In our study the serum progesterone level started to fall between days 15 and 20 to 21 after conception, and

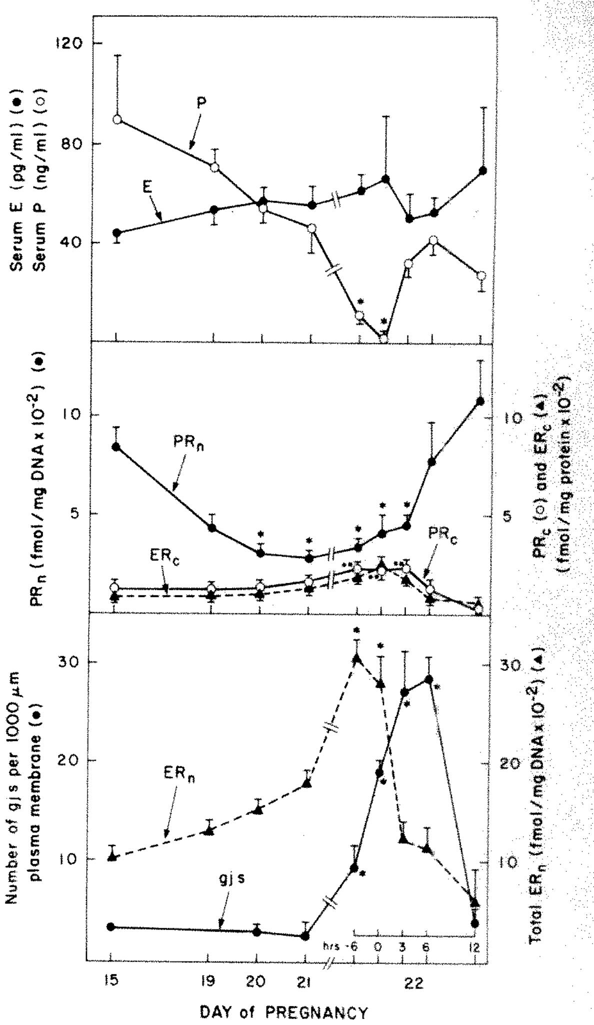

Fig. 3. Serum steroid levels, myometrial steroid receptor concentrations ($n = 3$ to 5, all values are mean ± SEM), and the number of gap junctions (*gjs*) during the latter half of pregnancy and after delivery. Serum progesterone levels 6 hours prior to labor and during labor (*) were significantly lower than the rest ($p < 0.05$). Peripartum progesterone nuclear receptor levels were significantly lower than at any other time (days 15, 19, 22 [6 hr], 22 [12]) > (20, 21, 22 [−6], 22 [0], 22 [3]); underlined groups were not significantly different from each other; $p > 0.05$, Duncan's multiple range test. Higher levels of estradiol nuclear receptors and numbers of gap junctions were observed around the time of labor (days 15, 20, 21) < (22 [−6 hr]) < (22 [0]) < (22 [3], 22 [6]) > 22 [12]). Levels of estradiol cytosol receptors and progesterone cytosol receptors on day 22 (**) were significantly higher than those on days 15 to 21 or 6 and 12 hours postpartum.

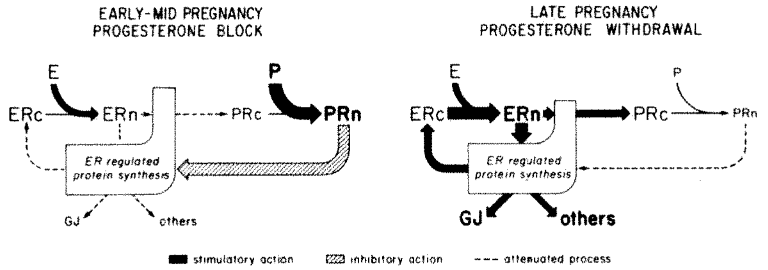

Fig. 4. Interrelationships of extracellular estrogen *(E)*, progesterone *(P)*, and their cytoplasmic (*ERc* and *PRc*) and nuclear (*ERn* and *PRn*) receptors during pregnancy and their roles in the control of estradiol receptor–mediated actions. The size and heaviness of each factor represent the relative quantity present. *Progesterone block:* During mid to late pregnancy, high circulating progesterone continuously enhances progesterone cytosol receptor translocation and maintains high levels of progesterone nuclear receptors. This dominance of progesterone nuclear receptors suppresses genomic expression of estradiol nuclear receptors, including induction of gap junctions *(GJ)* and progesterone cytosol receptors. Therefore the latter represents a suppression mechanism that imposes autoregulatory negative feedback on further or excess rise in the progesterone nuclear receptors. Thus at this stage the high circulating levels of estradiol are restrained in their "estrogenic" actions. *Progesterone withdrawal:* As labor approaches, levels of extracellular progesterone as well as progesterone nuclear receptors start to fall, resolving their inhibition of the *E→ERc→ ERn* system. Enhanced replenishment in estradiol cytosol receptors available to the circulating estradiol increases levels of estradiol nuclear receptors, even though the circulating estradiol does not show a marked peak at parturition. Consequently, estradiol nuclear receptor–dependent protein synthesis is turned on to increase gap junction formation, enzymes, receptors, secretory products, constitutive proteins, or prostaglandin synthesis, all of which are known to occur in an integrated manner at term in various species. The high level of estradiol nuclear receptors, however, also increases induction of progesterone cytosol receptors, and a rise in the circulating progesterone during the immediate postpartum period couples with this increasing level of progesterone cytosol receptors to cause accelerated translocation of progesterone cytosol receptors. The high level of progesterone nuclear receptors results in a myometrial condition more like the prepartum condition by blocking the genomic expression of estradiol effects.

a marked drop was observed in the morning of day 22. Although there appeared to be a gradual rise in estradiol level preceding labor,[2] we found considerable variance, which may reflect the higher sensitivity of the assay due to the use of ^{125}I-estrogen as a tracer ligand. In any case, without a marked change in serum estradiol level and the marked drop in serum progesterone level as pregnancy advanced, there was an increasing estradiol/progesterone ratio in serum in our study, as has been seen by others.[12] This more complete picture suggests that blood levels of steroids in the pregnant rat may not be an accurate indicator of steroid action or even tissue concentrations in this regard. Our work confirms this disparity as far as uterine steroid receptors are concerned. Myometrial progesterone receptors reached their nadir at least 48 hours before the fall of serum progesterone level was completed on day 22, showing that changes in the circulating steroid levels do not necessarily parallel changes in tissue receptors, especially the nuclear steroid receptor levels. In situ metabolism of progesterone may play a role in further disengaging tissue receptors from blood steroid levels. Substantial increases in 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one have been reported in uterine tissue between days 11 and 20 after conception.[13] In addition, evidence of local estrogen metabolism by the uterus has also been presented.[14]

While the concentrations of steroid hormone receptors are mainly influenced by the extracellular hormonal milieu, there is an important interplay in receptor control between estradiol and progesterone (Fig. 4). Estrogen stimulates replenishment of estradiol cytosol receptors, accelerates estradiol receptors translocation to the nucleus,[15] and induces progesterone cytosol receptors. In turn, the increase of progesterone cytosol receptors in the presence of progestin priming increases progesterone nuclear receptors and antagonizes replenishment of estradiol cytosol receptors in the rat uterus.[16] It is likely that the fall in serum progesterone, which occurs several days before the onset of labor, permits estradiol interaction with its receptors and enhanced translocation of estradiol nuclear receptors, thereby accelerating estradiol action on myometrium immediately before parturition.

The subtle but significant increase of levels of progesterone nuclear receptors between day 21 and the time of labor could be due to the rapid accumulation of estradiol nuclear receptors and the resultant progesterone receptor induction. Proportional increases in both estradiol cytosol receptors and estradiol nuclear receptors followed by an increase in oxytocin receptor have been reported[12] in the myometrium prior to parturition in the rat. Our data confirm the increase of estradiol receptors in both subcellular compartments.

Levels of estradiol nuclear receptors began to rise before the number of gap junctions increased. Since gap junction formation is estradiol responsive and also depends on protein synthesis,[3, 4] it is likely that estradiol nuclear receptor–mediated transcription and protein synthesis during the preceding 12 to 24 hours caused the offset of the gap junction curve and the estradiol nuclear receptor curve shown in Fig. 3. A closer analysis based upon more time points will be necessary to more clearly relate estradiol nuclear receptor to gap junction formation.

Present evidence from studies on reproductive organs, such as the uterus and ovary, indicates a possible involvement of prostaglandins or peptide hormones in increased size and number of gap junctions; however, estradiol seems to be the *primum movens* for inducing or increasing gap junctions. On the other hand, steroids, such as androgens and progestins, and nonsteroidal factors, such as catecholamines, are capable of diminishing gap junctions.[3, 4]

Besides the mechanism producing the gap junction network between myometrial cells at term, there seems to be a process that resolves myometrial gap junction during the postpartum period. Provided that high estrogenic conditions are maintained by exogenous estradiol administration, applied mechanical factors such as tension are documented to maintain the number of gap junctions even after labor.[16] While the rapid resolution of gap junctions within 12 hours post partum may be due to the decline of levels of estradiol nuclear receptors and to the release from tension, the postpartum increase in progesterone and progesterone receptors observed in our study might also serve as a gap junction–resolving factor, as is the case in in vitro experiments.[4]

Although studies in women have not shown a clear change in serum progesterone at term, many animal studies support Csapo's wisdom in identifying progesterone-related events as a major factor in the control of labor. For example, in certain animals, including rats, prepartum serum progesterone "withdrawal" plays a key role in onset of labor.[17, 18] Treatment of such animals with progesterone has been shown to delay the onset of labor effectively if progesterone is given as late as in the morning of day 21 but not after. The disappearance of uterine progesterone uptake in vivo prior to parturition associated with a diminution of cytoplasmic progesterone–binding activity has also been reported.[11] The present work indicates that autoregulatory mechanisms between the uterine estradiol receptors and progesterone receptors are an important link between circulating tissue levels of hormones and their genomic expression that controls labor (Fig. 4).

Taken together, it appears that the progesterone withdrawal theory can now be extended to include the following events: (1) during mid-late pregnancy the circulating estradiol level remains relatively constant, while the circulating progesterone level falls; (2) the level of progesterone nuclear receptors also falls, mirroring the circulating progesterone level; (3) with the fall in the level of progesterone nuclear receptors, the level of estradiol nuclear receptors rises, because of the persistent high serum estradiol level; and (4) the increased translocation of the estradiol/estradiol receptor complex induces formation of gap junctions.

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Peripartum colloid osmotic pressure changes: Effects of controlled fluid management

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A prospective, fluid-controlled study of serially measured colloid osmotic pressure changes in the peripartum period was undertaken. Seventeen patients with uncomplicated pregnancies undergoing elective cesarean section at term were administered a predelivery bolus of 15 ml/kg of lactated Ringer's solution prior to operation. Maintenance crystalloid fluids were infused at 125 to 150 ml/hr both intraoperatively and post partum without the addition of blood or other colloid solutions. Serial colloid osmotic pressure measurements were obtained before hydration, after hydration, after delivery, and at 6 and 24 hours post partum. The results demonstrated a 15.9% decline in colloid osmotic pressure immediately following the hydration bolus (20.7 ± 1.5 to 17.4 ± 1.8 mm Hg) ($p < 0.01$). A further decline in colloid osmotic pressure to 16.6 ± 1.7 mm Hg occurred after delivery and represented an overall 22% decrease from the baseline value ($p < 0.05$). The lowest mean colloid osmotic pressure value occurred at 6 hours post partum (16.1 ± 1.1 mm Hg). These data support previous observations that colloid osmotic pressure is uniformly lowered in the immediate postpartum period with peak reductions identified at 6 hours following delivery. In addition, intravenous crystalloid administration during the peripartum interval can substantially influence this decline in colloid osmotic pressure. Although no clinical evidence of cardiopulmonary compromise was observed in this set of normal gravid women, these data may be useful in the management of the parturient patient with established risk factors for pulmonary edema where alterations in the pulmonary capillary wedge pressure–colloid osmotic pressure gradient have been shown to correlate with the development of this complication. (AM J OBSTET GYNECOL 1985;151:812-5.)

Key words: Pregnancy, colloid osmotic pressure, hydration

In the late 1800s, Starling[1] first described the mechanism by which fluids transverse capillary membranes. With the recent advent of new technologies to measure the various components of Starling's equation more directly, renewed interest has developed in these variables as they relate to pulmonary edema. Previous investigators have shown that pulmonary edema is correlated with alterations in the pulmonary capillary wedge pressure to colloid osmotic pressure gradient.[2, 3] Similar relationships occur in pregnant patients and may be important in the production of pulmonary edema seen in the compromised gravid woman.[4] We and others have demonstrated that one component of this relationship, colloid osmotic pressure, is consistently lowered in the immediate postpartum period.[4-6] Iatrogenic instillation of crystalloid fluid has been shown in retrospective analyses to be a critical component in this reduction in oncotic pressure.[5, 6] Since pulmonary compromise is frequently identified during the immediate postpartum period, a better understanding of sequential changes in colloid osmotic pressure during this time frame may be important. This study

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was undertaken to define more precisely these peripartum colloid osmotic pressure changes prospectively under controlled fluid management.

Material and methods

Seventeen patients at term undergoing elective cesarean section at the University of Texas Medical School at Houston were enrolled in this prospective research study. The protocol was approved by the Committee for the Protection of Human Subjects at this institution. After written informed consent was given, baseline colloid osmotic pressure measurements were obtained. All patients were subsequently administered 15 ml/kg of lactated Ringer's solution as a prehydration bolus prior to initiation of anesthesia. Ten minutes after completion of this infusion, an additional colloid osmotic pressure measurement was determined. The type of anesthetic used for operation was at the discretion of the attending anesthesiologist.

During cesarean section, baseline intravenous crystalloid fluids were maintained at 125 to 150 ml/hr. In the event of hypotension as a result of the sympathetic blockade from conduction anesthesia, intermittent doses of intravenous ephedrine were used to control blood pressure. Blood loss was clinically estimated and blood was replaced intraoperatively, after the first 500 ml, with 2 to 3 ml of crystalloid per milliliter of blood loss. No colloids or blood products were used in any of these patients during the study period. In addition, patients experiencing excessive blood loss during the operative procedure were excluded from analysis. Additional colloid osmotic pressure measurements were taken immediately after operation and at 6 and 24 hours following operation. Postpartum intravenous fluids were maintained at 125 to 150 ml/hr.

Blood specimens were obtained in ethylenediaminetetraacetic acid–containing tubes, centrifuged, and the plasma decanted. Colloid osmotic pressure was measured on an IL-186 Weil oncometer. The mean of three determinations agreeing within 1 mm Hg was reported. A control of 5% albumin was measured every twentieth determination. The mean (±SD) for the control was 18.2 ± 0.2 mm Hg. Analysis of the data was by paired and unpaired Student's *t* test; significant levels were taken as $p < 0.05$.

Results

Seventeen gravid women at greater than 37 weeks' gestation underwent elective repeat cesarean section. None of the study patients' prenatal courses were complicated by hypertension, renal disease, or proteinuria. Ten patients were administered a general anesthetic and seven received a conduction anesthetic during this procedure. The mean volume of the prehydration fluid bolus given was 1079 ± 208 ml of lactated Ringer's solution. During operation, the mean volume of crystalloids administered was 1048 ± 323 ml. During the postpartum period all infusions were maintained at a designated intravenous rate of 125 to 150 ml/hr. A retrospective analysis of these intravenous fluids administered demonstrated the total volume during 24 hours to be 3106 ± 632 ml or an average of 129 ml/hr for the study period.

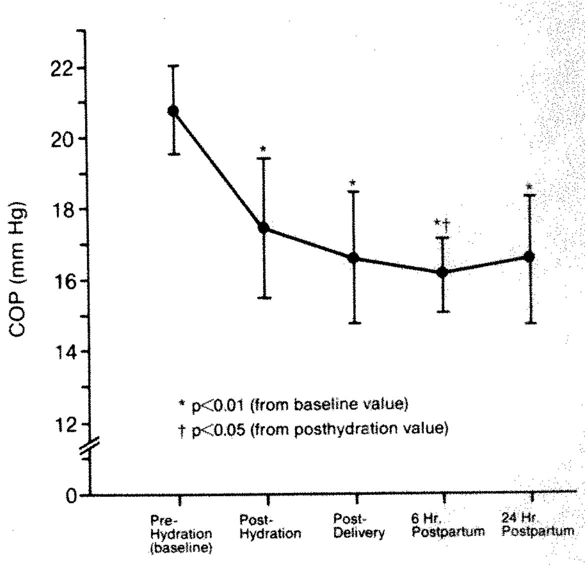

Fig. 1. Peripartum colloid osmotic pressure *(COP)* in patients undergoing cesarean section (n = 17). Values are mean ± SD.

The mean (±SD) colloid osmotic pressure values for the study patients are presented graphically in Fig. 1. The baseline colloid osmotic pressure for the entire group was 20.7 ± 1.5 mm Hg. Following the prehydration fluid bolus, this value declined to 17.4 ± 1.8 mm Hg ($p < 0.01$), a decrease of 15.9%. Immediately following operation, a further decline in colloid osmotic pressure to 16.6 ± 1.7 mm Hg was noted although this was not significantly different from the posthydration value. The lowest colloid osmotic pressure determination for the group was noted at 6 hours post partum, at which time the value was 16.1 ± 1.1 mm Hg. This measurement represented an overall 22% decline from the baseline value and was significantly lower ($p < 0.05$) than both the prehydration and posthydration values. Finally, at 24 hours, an upward trend was observed with a small rise in the colloid osmotic pressure to 16.5 ± 1.7 mm Hg for the group. Table I demonstrates the colloid osmotic pressure patterns observed in those patients under general and conduction anesthesia. No significant differences can be appreciated between these groups.

The lowest colloid osmotic pressure measurement recorded during our observation period was 13.4 mm

Table I. Colloid osmotic pressure changes in patients undergoing cesarean section while under general or conduction anesthesia

	Colloid osmotic pressure (mm Hg)	
Time of determination	*General anesthesia (n = 10)*	*Conduction anesthesia (n = 7)*
Before hydration (baseline)	20.8 ± 1.7	20.7 ± 1.2
After hydration	17.3 ± 1.7	17.5 ± 2.1
After delivery	16.8 ± 1.8	16.3 ± 1.7
6 hr post partum	16.1 ± 1.3	15.9 ± 0.9
24 hr post partum	16.3 ± 2.0	16.8 ± 1.1

Values are mean ± SD.

Hg (from a baseline value of 19.3 mm Hg) and occurred immediately after the prehydration bolus. At 6 hours post partum, this value rose slightly to 13.7 mm Hg and was 16.3 mm Hg by 24 hours. Overall, 10 of 51 (19.6%) postpartum colloid osmotic pressure values in six patients fell below 15 mm Hg during the study period. No patient in this study developed clinical evidence of pulmonary edema or signs and symptoms compatible with fluid overload.

Comment

Peripartum fluxes in intravascular fluids appear to play an important role in the pathogenesis of pulmonary edema.[7] Colloid osmotic pressure, one of the major factors opposing the egress of fluid into the interstitial space, has previously been shown to decline consistently post partum.[4, 5] Our results demonstrate that, in the patient with uncomplicated pregnancy undergoing cesarean section and receiving traditional volumes of crystalloid fluid, colloid osmotic pressure declines to its lowest levels (22% from baseline) by 6 hours following delivery. Furthermore, prehydration alone prior to induction of anesthesia can diminish colloid osmotic pressure values by 15.9%. This hydration-related decline in colloid osmotic pressure is similar to that reported previously in nonpregnant patients.[8] Additionally, these effects on colloid osmotic pressure have not been shown to be related to type of anesthesia or mode of delivery.[5] The results presented here are in agreement with these previously reported findings.

It is difficult to define the role of prophylactic maintenance of colloid osmotic pressure in the normal gravid woman. Anecdotal experiences along with controlled studies suggest that colloid osmotic pressure can be substantially reduced without overt clinical sequelae. However, when more carefully scrutinized, even these normal patients may show some evidence of incipient cardiopulmonary compromise.[9]

In the gravid woman with established risk factors for pulmonary edema,[10-12] a clinical algorithm incorporating some combination of the following three factors may have merit. First, accurate monitoring of crystalloid fluid administration should be the cornerstone of management in these "at risk" patients. In our experience the injudicious use of intravenous hydration is the most apparent reason for significant drops in colloid osmotic pressure during the immediate peripartum period.[5, 6] Second, colloid administration may be used to maintain colloid osmotic pressure levels in certain patients provided adequate renal function is demonstrable. Ramanathan et al.[13] have demonstrated that colloid infusion can prevent substantial declines in colloid osmotic pressure in the immediate postinfusion period. However, since these beneficial effects were not long-lasting, repeated dosing of colloid solutions may be required. Last, in those patients with severe pregnancy-induced hypertension, invasive hemodynamic monitoring can be used to monitor pulmonary capillary wedge pressure fluctuations more closely. Several investigators[2-4] have previously pointed out that significant alterations in the pulmonary capillary wedge pressure–colloid osmotic pressure gradient may be etiologic in the development of pulmonary edema. Both diuretics and afterload reduction agents can be used to influence this pressure gradient relationship in the pulmonary vasculature.[7] Monitoring this relationship may be particularly important in toxemic patients undergoing endotracheal intubation. Rafferty and Berkowitz[14] have shown that in these patients marked elevations in pulmonary capillary wedge pressure following endotracheal intubation can occur and may persist for up to 8 hours post partum. As demonstrated in our study, this unfortunately correlates with peak reductions in colloid osmotic pressure, which suggests that this time period be the most carefully watched.

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Ovine fetal urine production following maternal intravenous furosemide administration

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The response of the ovine fetus to maternal furosemide administration was studied in six chronically catheterized fetal lamb preparations. These studies indicate that in the chronic sheep model maternally administered diuretics do not augment fetal urine production. Additionally, passage of the drug from the maternal intravascular compartment to the fetal intravascular compartment could not be demonstrated. It is suggested that on the basis of these data, the results of the "Lasix challenge test" should be interpreted with caution when they are used to evaluate human fetal renal function. (AM J OBSTET GYNECOL 1985;151:815-9.)

Key words: Urine, furosemide, fetus, pregnancy

In 1973, Campbell et al.[1] reported a method for the antenatal measurement of human fetal urine production with the use of dynamic real-time ultrasound. This method is based upon serial measurement of three diameters of the fetal bladder. From these measurements bladder volume is derived. Change in fetal bladder volume over time is a reflection of fetal urine production.

Using the technique of Campbell et al., Wladimiroff[2] has reported the effects of maternal intravenous furosemide administration on human fetal urine production. This author reported that furosemide increased human fetal urine production by between 80% and 150% of pretreatment levels.

A recent study on fetal bladder volume determination with the use of real-time ultrasound has cast some doubt on the accuracy of this technique for the measurement of human fetal urine production.[3] In the above study fetal bladder volume was determined with the use of real-time ultrasound by two independent observers at half-hour intervals in nine healthy term fetuses for a 24-hour period. A total of 430 paired observations of fetal bladder volume were made. While no statistically significant difference in mean fetal bladder volume was found between observers, a wide standard deviation was noted. Additionally, large variations (>10 ml) between observers were noted in 19.1% of

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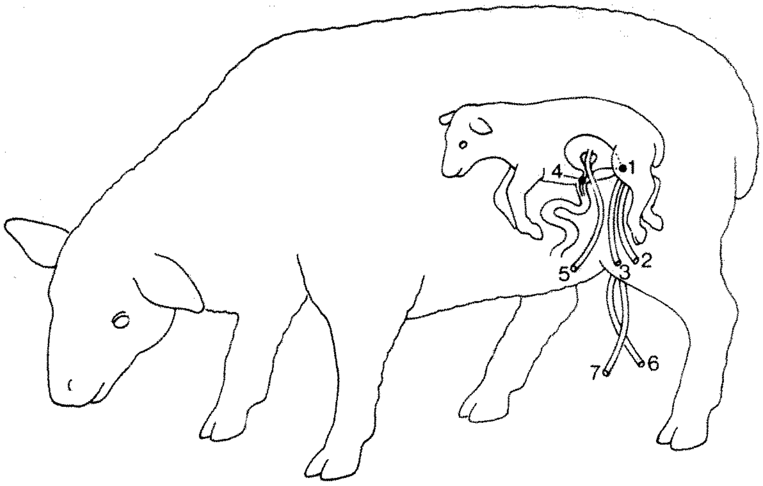

Fig. 1. Schematic representation of the animal model. *1* = Urethral ligation; *2* and *3* = fetal femoral arterial and venous catheters; *4* = urachal ligation; *5* = fetal bladder catheter; *6* and *7* = maternal femoral arterial and venous catheters.

paired observations when individual measurements of fetal bladder volume were considered.

In clinical high-risk obstetrics, furosemide has received increasing popularity in the antenatal assessment of fetal renal function. The so-called "Lasix challenge test" is frequently used to promote fetal bladder filling in cases where either oligohydramnios is noted or the fetal bladder is not visualized at the time of real-time scanning.[4, 5] A positive test is recorded when the fetal bladder is visualized following furosemide administration. This test is thought to aid in differentiation between the structurally normal fetus with oligohydramnios and the fetus with renal agenesis and oligohydramnios.

Prompted by the above reports and by our own observations on the inaccuracy of fetal bladder volume determination with the use of dynamic real-time ultrasound, we have undertaken a study of the effects of maternal intravenous furosemide administration on fetal urine production in the chronically catheterized ovine fetus.

Material and methods

Pregnant mixed-breed ewes of known gestational age ($\geq$120 days) were used for all experiments.

Surgical preparation. The ewes were anesthetized with a 4% Halothane–100% oxygen mixture administered via a nose cone. Following induction of anesthesia, endotracheal intubation was performed and anesthesia was maintained with a 1.5% Halothane–100% oxygen mixture. The animals were restrained in a supine position and the abdomen shaved and cleansed in a standard surgical fashion. Through a left groin incision the maternal femoral artery and vein were catheterized. Midline vertical lower abdominal laparotomy was then performed.

A fetal hind limb was identified through a cotyledon-free space in one uterine horn and a purse-string suture inserted into the uterine wall. Hysterotomy with delivery of the fetal hind limbs and rump to a level midway between the symphysis pubis and the umbilical cord insertion was performed. Via a suprapubic transverse incision the fetal urethra was identified and doubly ligated. Lower abdominal midline vertical laparotomy midway between the symphysis pubis and umbilical cord insertion was then performed. The umbilical arteries and urachus were identified and the urachus mobilized. A 7-gauge Swan-Ganz catheter was inserted into the fetal bladder via the urachus and the balloon inflated with 1.5 ml of air. Urachal ligation distal to the catheter insertion was performed and the fetal abdominal wall repaired. Finally, the fetal femoral artery and vein were catheterized via a left or right groin incision (Fig. 1). Following catheter placement the fetus was returned to the uterine cavity and the hysterotomy incision repaired by ligation of the purse-string suture. Both maternal and fetal catheters were mobilized to the exterior through a left maternal flank incision using a subcutaneous tunnel. The maternal abdominal and groin incisions were then closed.

Postoperative care

Ewe. At the time of completion of the surgical preparation 60 mg of gentamicin and one million units of penicillin were given intravenously to the ewe and the maternal vascular catheters flushed with heparinized saline (1000 units of heparin per 500 ml of normal saline solution). The antibiotics were repeated daily for 3 days.

Fetus. Gentamicin, 20 mg, and penicillin, 500,000 units, were administered intravenously to the fetus at the time of completion of the surgical preparation and the fetal vascular catheters flushed with heparinized

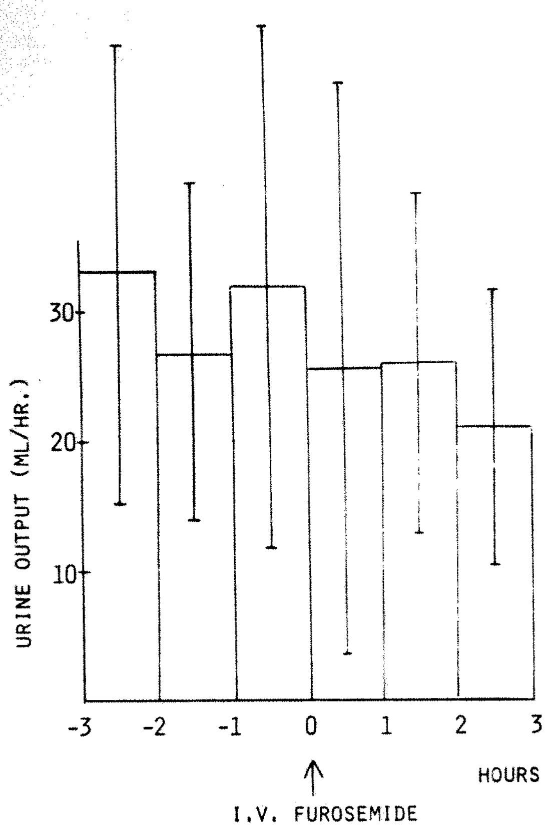

Fig. 2. Fetal urine output following furosemide, 1 mg/kg of maternal weight, to ewe (n = 6, mean ± SD). Mean hourly fetal urine output: Before furosemide, 30.7 ± 17.4 ml (SD); after furosemide, 24.5 ± 16.1 ml (SD) (not significantly different).

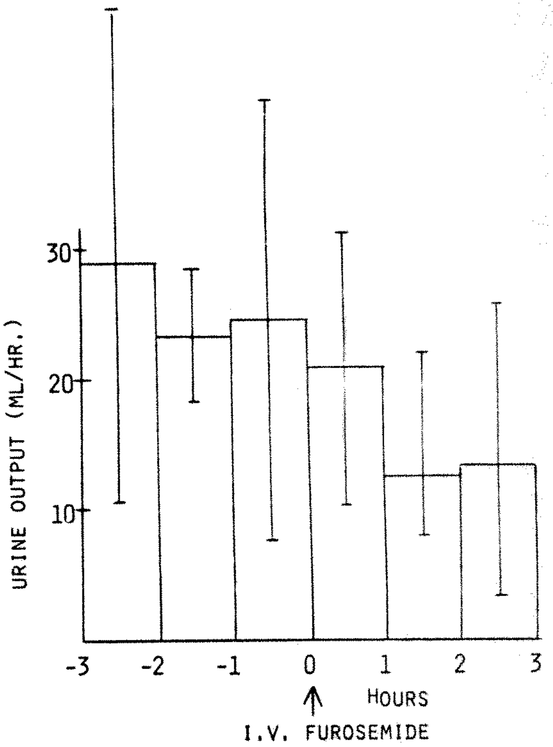

Fig. 3. Fetal urine output following furosemide, 2 mg/kg of maternal weight, to ewe (n = 6, mean ± SD). Mean hourly fetal urine output: Before furosemide, 25.8 ± 17.1 ml (SD); after furosemide, 15.6 ± 11.0 ml (SD) ($p < 0.05$).

saline solution. Penicillin, 500,000 units intravenously, was repeated daily for 3 days.

Both maternal and fetal vascular catheters were flushed daily with heparinized saline solution and arterial blood was obtained for maternal and fetal pH and blood gas determination. The fetal urinary catheter was connected to a collection bag attached to the left maternal flank and the urine output recorded.

Diuretic protocol. All preparations were allowed a minimum of 72 hours after operation prior to being studied. The diuretic protocol was as follows: (1) baseline hourly fetal urine production was determined for 3 hours prior to diuretic administration by direct measurement. (2) Furosemide in doses of either 1 or 2 mg/kg of maternal weight was then administered intravenously as a bolus to the ewe (dosage range: 65 to 155 mg). (3) Maternal and fetal venous blood samples were obtained before and at 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after diuretic administration for furosemide assay. (4) Hourly fetal urine production was recorded for the 3 hours after diuretic administration and urine samples collected for fetal urinary osmolarity determination. (5) Normal saline solution with supplemental potassium chloride (20 mEq/L) was administered intravenously to the ewe following the diuretic bolus to ensure adequate maternal hydration as follows: (a) furosemide, 1 mg/kg of maternal weight—1 L during 3 hours; (b) furosemide, 2 mg/kg of maternal weight—2 L during 3 hours.

Biochemical analysis. Urinary osmolarity was measured with an Advanced osmometer, Model 3L (Advanced Instruments, Needham Heights, Massachusetts). Furosemide levels were measured by the modified high-pressure liquid chromatography method of Nation et al.[6]

Data analysis. The mean and SD for hourly fetal urine production and fetal urinary osmolarity were calculated for each of the 3 hours before and after diuretic administration. Statistical significance was determined by Student's *t* test if p values of ≤ 0.05 were found.

Results

The effects of maternal intravenous furosemide administration on hourly fetal urine production and fetal urinary osmolarity were studied in six animal preparations between 120 and 140 days' gestational age. In all cases fetal pH and Po_2 were >7.3 and >15 mm Hg, respectively, at the time of diuretic administration. The results of these experiments are indicated graphically in Figs. 2 to 4.

Hourly fetal urine production. Mean hourly fetal

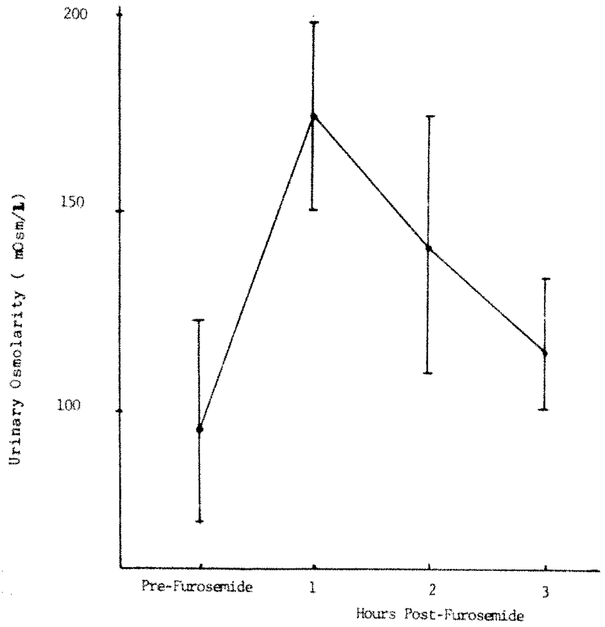

Fig. 4. Fetal urinary osmolarity following furosemide, 2 mg/kg of maternal weight intravenously to ewe (n = 6, mean ± SD). Mean fetal urinary osmolarity: Before furosemide, 92 ± 32 mosm/L; 1 hour after furosemide, 175 ± 26 mosm/L ($p < 0.05$).

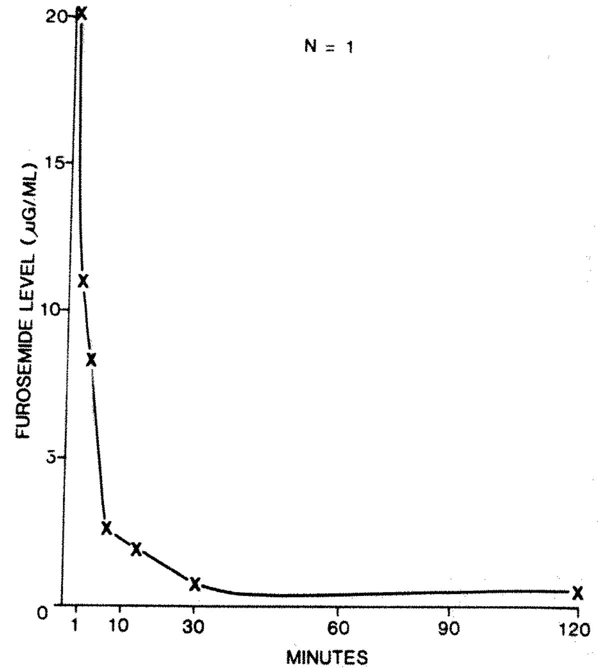

Fig. 5. Decline in maternal venous plasma furosemide levels following furosemide, 2 mg/kg of maternal weight intravenously to ewe.

urine production fell following maternal intravenous furosemide administration. In the experiment with furosemide, 1 mg/kg of maternal weight, mean hourly fetal urine production before furosemide administration was 30.7 ± 17.4 ml (SD). Mean hourly fetal urine production after furosemide was 24.5 ± 16.1 ml (SD) (Fig. 2). This difference is not statistically significant.

In the experiment with furosemide, 2 mg/kg of maternal weight, mean hourly fetal urine production before furosemide was 25.8 ± 17.1 ml (SD). Mean hourly fetal urine production after furosemide was 15.6 ± 11 ml (SD) (Fig. 3). This difference is statistically significant ($p < 0.05$).

Fetal urinary osmolarity. The change in fetal urinary osmolarity following the administration of furosemide, 2 mg/kg of maternal weight, to the ewe was studied in six animal preparations. Fetal urinary osmolarity was noted to increase significantly in the first hour after furosemide administration (Fig. 4). The mean fetal urinary osmolarity before furosemide was 92 ± 32 mosm/L. Mean fetal urinary osmolarity 1 hour after furosemide was 175 ± 26 mosm/L. This difference is statistically significant ($p < 0.05$).

Fetal and maternal venous plasma furosemide assays. Furosemide was not detected at any time during the first 3 hours following maternal intravenous furosemide administration in fetal venous plasma. The decline in maternal venous plasma furosemide levels following an intravenous bolus of 2 mg/kg of maternal weight is shown in Fig. 5. A rapid decline in maternal venous plasma furosemide levels occurred during the first 30 minutes following intravenous furosemide administration.

Comment

The results of this study indicate that in the ovine fetus maternally administered intravenous furosemide does not augment fetal urine production. Rather, the converse is noted with a fall in fetal urine production following maternal intravenous furosemide administration. It is of interest to note that the larger the dose of the diuretic given, the greater the fall in fetal urine production. Additionally, the response in fetal urinary osmolarity was converse to that which would be expected with this diuretic. Finally, in this study we were unable to demonstrate transport of furosemide from the maternal intravascular compartment to the fetal intravascular compartment.

The results of this study were unexpected and suggest that the ovine fetus responds to maternal intravascular volume depletion by fluid retention and a decrease in its urine flow. The mechanism by which this response occurs is unknown but may indicate an antidiuretic hormone response on the part of the fetus (W. Jacyk, M.D., personal communication). Further studies into this mechanism are presently ongoing in this institution.

Our failure to identify furosemide in fetal venous plasma may be explained by one of several possibilities. Furosemide is poorly soluble in both water and lipid, and, despite its low molecular weight (331), it is a highly charged molecule.[7, 8] Additionally it is strongly bound to plasma proteins. These factors make transfer of the drug across the placenta unlikely.[9] The furosemide assay that we used in this study is sensitive to a concentration of 100 ng/ml. It is possible that transfer of the drug occurred to a level less than the sensitivity of our assay and hence was not detected. We feel this possibility to be unlikely.

As part of this study protocol, direct intravenous administration of furosemide to the fetus was also performed. Preliminary observations suggest that, in order to prompt a fetal diuresis, direct administration of furosemide in doses >3 mg/kg of estimated fetal weight are required. Direct intravenous boluses of this magnitude caused a significant increase in fetal urine production in some but not all animal preparations (P. F. Chamberlain, M.B., unpublished observations).

In view of the findings of this study, we feel that the results of the "Lasix challenge test" should be interpreted with caution when used for evaluation of human fetal urine production. The application of data obtained from the chronic animal preparation to the human setting is not absolute as differences in drug transfer across the placenta are known to exist between species (M. Harrison, M.D., personal communication).

However, it is possible that the previously reported augmentation in human fetal urine production attributed to furosemide may simply represent a spontaneous variation in bladder filling. Studies to investigate transfer of furosemide from the maternal intravascular compartment to the fetus in human pregnancy are presently under way in this institution. Until such information is available, and in view of the findings of this study in the chronic sheep model, failure to identify the human fetal bladder following maternal furosemide administration should not be interpreted as an absolute indication of absent fetal renal function.

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Osmolarity changes can enhance the release of renin from human chorion

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Sections of human chorion were perfused with an aerated buffer for 5 hours. Samples were collected every 20 minutes and the concentration of active and total renin in the perfusate was measured. Substitution of the initial bicarbonate buffer (281 mosm/L) with a buffer of 251 mosm/L doubled the rate at which active and inactive renin were released into the perfusate. When the osmolarity of the solution was reduced by 60 mosm/L, a threefold rise in the concentration of active renin and a sixfold rise in that of total renin was seen. However, these effects were transient, and renin secretion decreased before osmolarity was raised to the previous value. (AM J OBSTET GYNECOL 1985;151:819-21.)

Key words: Fetal membranes, chorion, renin

The presence of renin in certain tissues of the human uterogenital tract[1] and the subsequent report of its synthesis in human chorion and myometrium[2] have led many scientists to speculate on the physiologic role of

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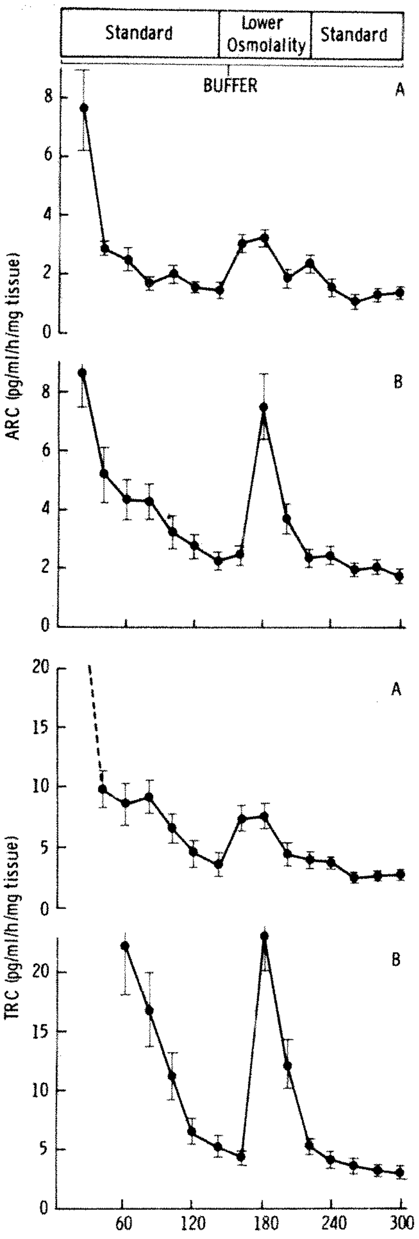

Fig. 1. Reducing the osmolarity of a standard bicarbonate buffer by 30 mosm/L *(A)* or 60 mosm/L *(B)* increases the rate of secretion of active renin concentration (ARC) and total renin concentration (TRC) from human chorion. Each *point* is the mean value from six experiments. The *vertical bar* represents the standard error of the mean.

the congener. Studies that have been conducted with animals have, in most cases, led to the conclusion that this renin is not released by stimuli that initiate the release of the renal enzyme. We have already shown that when prostaglandin E_2 is perfused through human chorion, the pattern of renin release does not change[3]—an observation that emphasizes the difference between chorionic renin and renal renin, because the latter is readily released by superfusion with the prostaglandin.[4]

Table I. Values of active renin expressed as a percentage of total renin

Time at which sample was collected (min)	*Change in osmolarity**	
	−30 mosm/L	*−60 mosm/L*
20	28.1 ± 8.9	24.1 ± 7.8
40	35.0 ± 6.4	28.3 ± 6.2
60	30.3 ± 3.6	37.0 ± 5.0
80	27.1 ± 5.9	40.0 ± 6.0
100	42.1 ± 7.1	46.3 ± 6.0
120	50.2 ± 8.7	50.8 ± 7.8
140	58.3 ± 10.3	60.9 ± 8.9
160	66.2 ± 9.9	69.8 ± 8.4
180	52.4 ± 7.3	38.1 ± 5.5
200	57.6 ± 8.9	62.2 ± 7.1
220	52.2 ± 6.1	58.1 ± 6.8
240	56.5 ± 9.4	65.5 ± 7.7
260	71.4 ± 8.7	63.6 ± 8.1
280	70.2 ± 10.5	73.1 ± 6.8
300	73.8 ± 8.4	66.6 ± 8.4

*Percent of active renin concentration divided by total renin concentration. Values are means and standard errors of the mean of six samples.

The juxtaglomerular cell of the kidney (the source of renin) is a sensitive osmometer, since a reduction in the osmolarity of a superfusing solution by 50 mosm/L gives rise to a fivefold increase in the amount of renin that is released from these cells.[5] The authors who demonstrated this suggest that in vivo renin release might be controlled by the osmolarity of blood and renal interstitial fluid, which in turn would depend on the various solutes that are present in the two fluids. In view of the intimate relationship of the chorion with amniotic fluid and maternal blood, we have studied the effect of osmolarity on the release of active and inactive chorionic renin.

Material and methods

Placentas were collected from 12 normotensive women after spontaneous vaginal delivery. Chorion laeve was separated from amnion by means of the method adopted by Skinner et al.,[1] and this tissue was then placed in a bicarbonate buffer (in mmol/L: sodium chloride, 91.0; sodium bicarbonate, 17.5; potassium chloride, 7.0; calcium chloride, 2.0; magnesium sulfate, 1.2; monosodium acid phosphate, 1.2; glucose, 11.0; sucrose, 50.0) with pH of 7.4 at 4° C. Sections of the chorion laeve were placed in a Unipore 47 mm membrane filter holder (Bio-Rad Laboratories) and perfused via polyethylene tubing with a solution of the bicarbonate buffer (at 37° C, aerated with 95% oxygen and 5% carbon dioxide) at the rate of 0.25 ml/min.[3] Samples of the perfusate (at 4° C) were collected every 20 minutes. After 140 minutes a buffer of similar composition but containing either 25 mmol sucrose or no sucrose was perfused for 80 minutes. Following a further 80-minute period of perfusion with the original buffer, the chorion was dried on filter paper and was then weighed. The perfusate was stored at −20° C until it was assayed for active and total renin concentration.

The concentration of active renin was measured indirectly by radioimmunoassay of angiotensin I in the presence of excess sheep renin substrate as described by Craven and Symonds.[6] For the determination of total renin concentration, portions of the perfusate were dialyzed to a pH of 3.3 for 18 hours, were incubated at 32° C for 30 minutes, and were then dialyzed to a pH of 7.5 for 18 hours. The amount of renin in these samples was measured by means of the above procedure. The concentration of renin was expressed as picogram of angiotensin I generated per milliliter of perfusate per hour of incubation per milligram of tissue (wet weight).

The results were analyzed by paired and unpaired Student's t test. Significance was defined as a p value of <0.05.

Results

For the first 80 minutes of the perfusion the concentration of active and total renin in the samples gradually decreased to a level that was stable during the remainder of the experiment and which we accepted as a baseline. The percentage of active renin in these samples was significantly less than that in samples that were collected later ($p < 0.01$). The most likely explanation for such a difference during this "wash-out" period is that the ratio of inactive to active renin is higher in the interstitial spaces of the choriodecidua than it is in the intracellular areas.[3] Fig. 1 shows that reducing the osmolarity of the perfusing buffer by 30 mosm/L doubled the amount of active and inactive renin that was released into the perfusate—an increase that was statistically significant ($p < 0.02$). A reduction from 281 mosm/L to 220 mosm/L resulted in a threefold rise in the concentration of active renin and a sixfold rise in that of total renin. It is interesting to note that this augmented release of renin is not sustained and gradually returns to normal while the buffer of lower osmolarity is being perfused. A reduction in osmolarity in the juxtaglomerular cells of the kidney also leads to a transitory increase in the release of renin.[5] Perfusion with the 220 mosm/L solution releases proportionately more inactive renin ($p < 0.02$), as Table I illustrates.

Comment

Although there is an inverse relationship between the osmolarity of a solution surrounding the juxtaglomerular cells of the kidney and the release of renin from these cells,[5, 7] the existence of such a correlation in the chorion has not been investigated. We have shown that a reduction in osmolarity enhances the release of renin from human chorion. In order to avoid the possibility that sodium ions may directly alter renin release,[5] we changed the osmolarity of the perfusing solution by lowering the concentration of sucrose. Since the report that sections of chorion that have been incubated in vitro are able to produce angiotensin I without the addition of renin substrate,[8] the suggestion of a local role for this renin-angiotensin system has been strengthened. We can, however, only speculate on which of the substances that are present in amniotic fluid and in maternal blood control the release of chorionic renin. Since lipids, phospholipids, cholesterol, creatinine, uric acid, urea, glucose, and electrolytes are constituents of the two fluids and since the concentration of each of these elements is seldom the same in both, the problem seems insuperable. Urea is not, however, likely to control the release of renin from chorion, because it has a weak osmotic effect.[7]

The reduction in osmolarity alters the ratio of active to inactive renin in favor of the latter. This result adds further proof to the belief that the latter is a "prorenin,"[9] and suggests that under certain circumstances there is a limit to the rate at which the inactive precursor is converted to the active enzyme.

We wish to thank the technical staff in the Department of Obstetrics and Gynaecology for their expertise and industry. Our thanks also go to the Medical Research Council for supporting Dr. S. F. Cooke by means of Medical Research Council Grant No. G979/813/SB and to Mr. Colin Selby of the Department of Biochemistry, City Hospital, Nottingham, England, for determining the osmolarity of the buffer solutions.

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Laminin distribution in human decidua and immature placenta

An immunoelectron microscopic study (avidin-biotin-peroxidase complex method)

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An immunoelectron microscopic study was carried out on human placenta and decidua with the use of preembedding, the avidin-biotin-peroxidase complex technique, and rabbit antimurine laminin antibody. Laminin was detected in the lamina lucida of basement membrane of placental villi, amniotic membranes, umbilical cord, endometrial glands, and blood vessels. No positive laminin immunostaining was observed in intracytoplasmic organelles. However, positive immunostaining surrounded decidual cells as a more or less continuous linear membrane. It is suggested that laminin, as a component of this basement membrane–like material that has already been reported in decidual cells, may be related to the hormonal stimulation occurring during pregnancy and trophoblastic attachment. (AM J OBSTET GYNECOL 1985;151:822-6.)

Key words: Placenta, laminin

Basement membranes are sheets of extracellular matrix that separate epithelia, endothelia, mesothelia, muscle fibers, adipocytes, and perineural cells from connective tissue. Several macromolecules including type IV collagen,[1, 2] proteoglycans,[3, 4] and noncollagenous glycoproteins, specifically entactin,[5, 6] laminin,[7-9] and fibronectin,[10] have recently been defined as basement membrane components. Laminin has been isolated from the extracellular matrix of cultured murine endodermal cells[8, 11] from the EHS sarcoma[9] and from the rat yolk sac tumor.[12-14] The glycoprotein is composed of disulfide bound subunits of 200,000 and 400,000 dalton molecular weights.[9, 14, 15]

Immunohistochemical studies have localized laminin in the embryonic endodermal cells and the Reichert's membrane of the rat parietal yolk sac,[16-18] in adult mouse and rat tissues,[7, 13, 14, 18] in the extracellular matrix and the tumor cells of the EHS sarcoma and of the rat yolk sac tumor,[12-14, 16] and in various human tissues.[7, 9, 19-22]

Among the studies dealing with human tissues very few have included placenta and decidua. Recently, however, Alitalo et al.[23] localized laminin in human amniotic membranes and cultured human amniotic cells. Also Wu et al.[6] localized laminin and entactin in mouse embryos and fetuses mainly in the extracellular membranes, endodermal cells, and "deciduoma." Wynn[24] described a "basement membrane–like material" around human decidual cells. The aim of the present study was to search for laminin in this basement membrane–like material in human decidua and placenta by means of immunoelectron microscopy.

Material and methods

Tissue preparations. Tissue obtained from 10 uterine aspirations of 8-week pregnancies was sampled for histologic evaluation. Fresh tissue was separated into three groups: decidua, placenta villi, and umbilical cord. Samples of each group were (1) Bouin fixed and paraffin embedded, (2) immediately frozen in the cryostat, (3) cut into 1 mm^3 fragments and fixed in 2% glutaraldehyde for electron microscopic examination, and (4) immediately cut into 2 mm slices, rapidly fixed for 75 min at 4° C in 4% paraformaldehyde, and cut with a Vibratome (Lancer 1000) into 100 μm thick sections before immunoperoxidase staining.

Antigen. Laminin was purified from the matrix of the EHS sarcoma[25] according to the technique described by Timpl and Rohde.[9] The antigen was further purified by affinity chromatography on lectin B4 extracted from Bandeiraea simplicifolia.[26]

Antisera. Antisera to murine laminin were prepared by immunizing rabbits by a subcutaneous injection of 250 μg of laminin in Freund's adjuvant and subsequent injections of 100 μg every 14 days. The animals were

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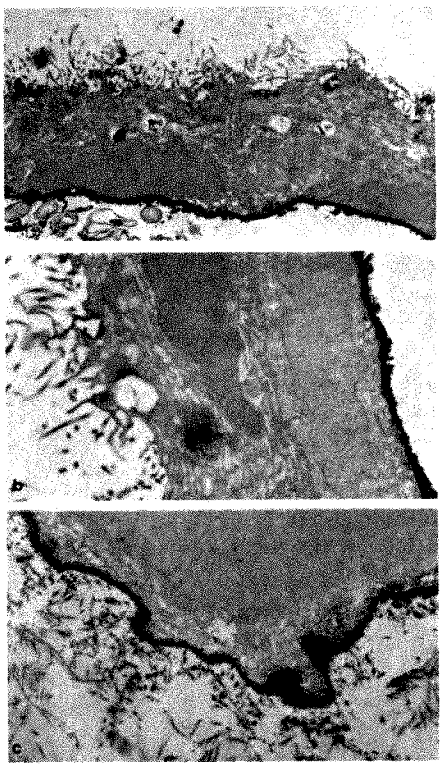

Fig. 1. Positive laminin immunostaining appears *(a)* beneath trophoblastic cells in placental villi (× 3000), *(b)* in basement membrane along trophoblastic cell plasma membrane (× 7000), and *(c)* in amniotic cells of umbilical cord (× 11,400). No positive staining is visible within cells.

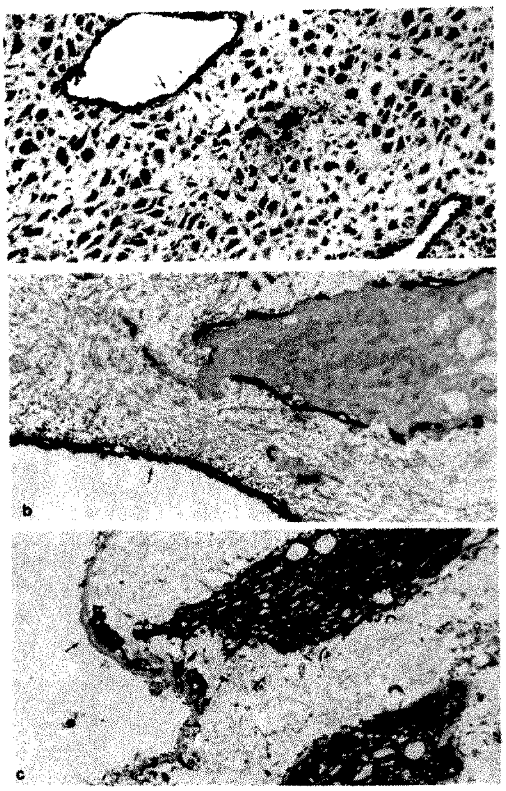

Fig. 2. *(a)* Decidualized endometrium with polygonal stromal cells, endometrial gland, and blood vessel (× 300); *(b)* positive laminin immunostaining around decidual cells in a basement membrane–like material and on the glandular basement membrane (× 7000); *(c)* negative control with rabbit serum devoid of antilaminin activity by adsorption to murine laminin (× 6400). *(Arrow:* gland).

bled every 2 weeks 1 week after each injection. The antilaminin activity was determined by means of a "dotting test" as reported by Hawkes et al.[27] The antiserum used for negative controls was obtained by adsorption of rabbit antimouse laminin to mouse laminin coupled to (1) Affigel 10, then (2) Sepharose 4B–heparin, and finally (3) Sepharose 4B–lectin B4. A dot-immunobinding assay revealed no residual laminin affinity on the eluted serum.

Immunostaining procedure. For the light microscopic study the immunoperoxidase technique was performed by means of the avidin-biotin-peroxidase complex technique (Vector Laboratories Inc., Burlingame, California) as previously described.[28, 29] In addition, some sections were submitted to trypsin digestion prior to the avidin-biotin-peroxidase technique.

For the electron microscopic study the avidin-biotin-peroxidase technique was performed on free-floating thick sections incubated in Petri dishes. Fragments of these sections were embedded in 25 Araldite blocks. Ultra-thin sections were cut with a diamond knife and collected on 300 mesh copper grids (a mean range of 5 grids for each Araldite block).

Controls. Tissue samples from murine sarcoma and normal human thyroid gland were used as positive tissue controls for light microscopic studies. Fresh normal human thyroid fragments and type I collagen film (CERAD, Lyon, France) coated with laminin (6 μg/mm^2) were used as positive tissue controls for electron microscopy. The specificity of the immunostaining procedure was controlled by substituting the specific antilaminin antibody with (1) phosphate buffer saline, (2) irrelevant antisera (anticasein, antithyroglobulin), and (3) laminin-adsorbed specific antiserum.

Results

Light microscopic examination. No positive laminin immunostaining was ever observed in paraffin sections even after trypsinization. In contrast, on unfixed frozen sections, positive laminin immunostaining was consistently observed within the basement membranes but never within the cytoplasm.

Immunoelectron microscopic examination. A better definition of the laminin distribution was obtained: positive osmiophilic dots were located in basement membranes under (1) the amniotic epithelium of the umbilical cord and of the amniotic membranes (Fig. 1), (2) the trophoblastic cells in placental villi (Fig. 1), (3)

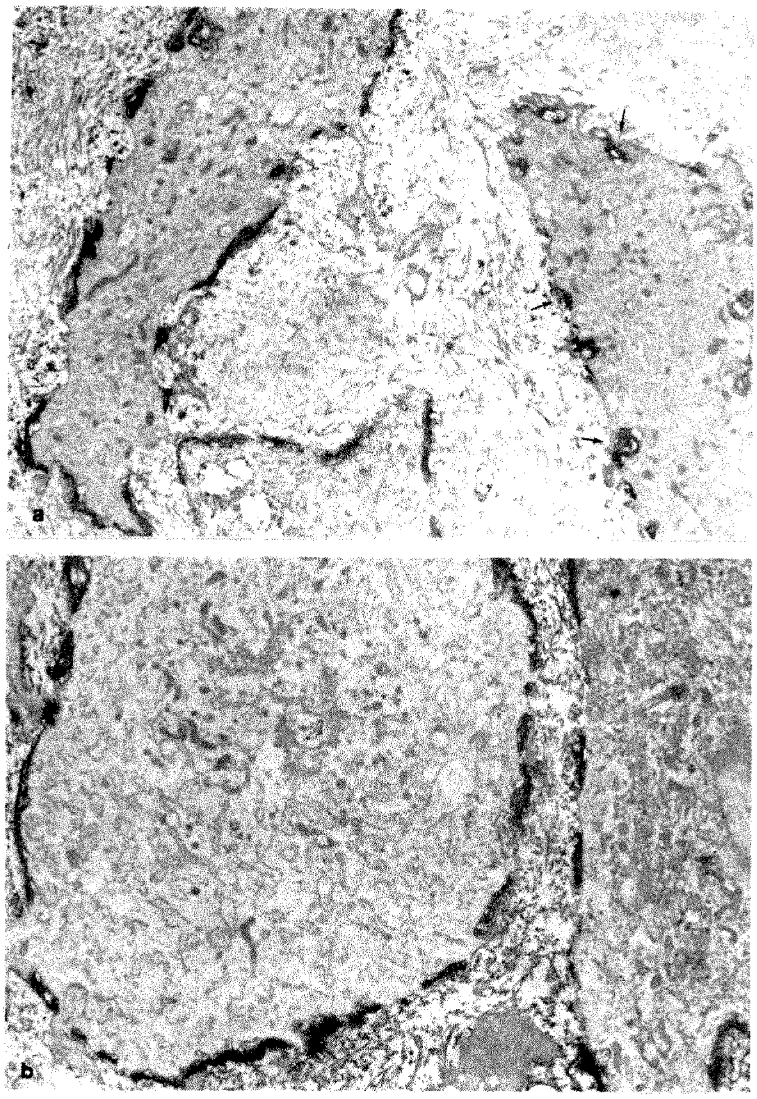

Fig. 3. Positive laminin immunostaining displays a more or less linear pattern along plasma membranes of decidual cells. Pattern of laminin liberation is visible in some areas *(arrows)* (×7000).

the glandular cells in decidua (Fig. 2), and (4) the endothelial cells of vessels. In addition, a highly consistent positive staining surrounded the large polygonal decidual cells. The cell borders were underlined by a thick, dark, often incomplete line corresponding to the pericellular basement membrane–like material observed in unstained control sections (Figs. 3 and 4). No laminin was detected in endoplasmic reticulum, Golgi apparatus, or secretory granules in any of the cells. However, in an occasional decidual cell, positive immunostaining appeared to be located in the most external part of the cytoplasm in continuity with the extracellular positivity (Fig. 3). In positive controls, immunostaining was observed in thyroid basement membranes and on the surface of collagen films coated with murine laminin. Conversely, no staining appeared in negative controls.

Comment

Several previous immunoelectron microscopic studies have shown that laminin is distributed in basement membranes either in the lamina densa or the lamina lucida.[7, 12, 13, 17, 18, 22] Our results indicate that laminin is situated either in lamina lucida or in both lamina lucida and lamina densa of human placental basement membrane, since there was no staining gap between the plasma membrane and the positive immunostaining. In contrast, with the use of the same technique, laminin was observed only in the lamina densa of human breast and thyroid (unpublished observations).

Very few studies have shown an intracellular localization of laminin.[16, 18] Laurie et al.[18] demonstrated the presence of laminin in the endoplasmic reticulum, the Golgi apparatus, and the secretory granules of rat endodermal cells. In human tissues the presence of lam-

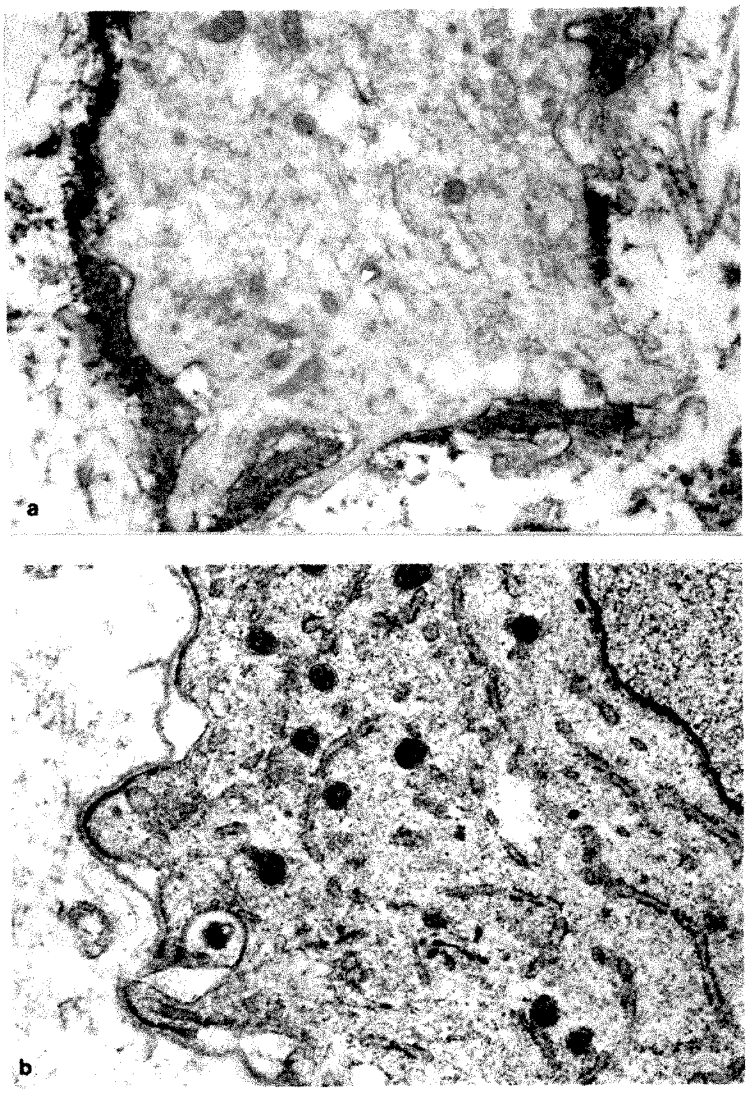

Fig. 4. Positive laminin immunostaining in *a* corresponds to basement membrane–like material *(b)* visible on negative controls after uranyl acetate–lead citrate staining (×26,000). It displays the pattern described in Fig. 13 of Wynn.[24]

inin has been reported in carcinoma cells.[30, 31] In our experience we never detected laminin within the cell organelles, either in immature placenta or in other human tissues such as thyroid, breast, pancreas, and endometrium. These discordant results may be due to a masking phenomenon of laminin antigenic sites or to the inherent properties of the different antibodies used. However, even after trypsinization we did not obtain any immunostaining improvement or intracellular positivity. Also the electron microscopic immunostaining was performed on sections that permitted positive cytoplasmic staining with other antisera (anticasein, antikeratin, antithyroglobulin) and other tissues (breast, thyroid) (unpublished observations). Therefore the absence of intracellular laminin staining could not be attributed to the failure of the reagents to penetrate tissues but may be related to the fact that our antiserum detects laminin only in its extracellular conformation. An occasional positive immunostaining appeared to be situated in the most external part of the cytoplasm of decidual cells in continuity with extracellular laminin immunostaining. These findings suggest that changes may occur in the molecular organization of laminin upon its liberation from cells, such as into the basement membrane–like material described by Wynn.[24]

This basement membrane–like material has never been reported in nondecidualized endometrium and is associated with specific morphologic changes of stromal cells caused by hormonal stimulation occurring during pregnancy.[24] These data suggest that laminin detection around decidual cells may also be related to this hormonal stimulation. Also, laminin has been shown to be an important factor for cell attachment[32] and therefore may be involved in the attachment of trophoblastic cells to endometrium during early pregnancy.

We are grateful to S. Reynaud and R. Gochgagarian for iconographic assistance. We are indebted to Pr. H. Ruf from la Maternité de la Belle de Mai, Marseille, for providing the uterine aspirations. We also thank A. Huc (CERAD, Lyon, France) for the collagen films.

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CORRESPONDENCE

Long-term reversible contraception with levonorgestrel-releasing Silastic rods

To the Editors:

The article entitled "Long-Term Reversible Contraception With Levonorgestrel-Releasing Silastic Rods" (Am J Obstet Gynecol 1984;148:1006), by Roy, Mishell, Robertson, et al., contains the following sentences without references to my original work in the invention, methods of manufacture, and uses of the Silastic rods. The sentences in question appear on page 1007. "Manufacture of the capsules is tedious, and placing and removing six capsules creates some difficulties." In 1972, I developed the Silastic rods because of the very problems mentioned by Roy, Mishell, Robertson, et al., as well as other problems not mentioned (Gupta GN. Silastic solid rods of contraceptive hormones. First reported in three progress reports under NIH-P01HD0567-01 support, 1972 to 1974.) "A new method of manufacturing implants has been devised." On this point there was again no reference to my work with the rods. (Gupta GN. Effects of estradiol-17β and its esters from new subcutaneous Silastic solid implants on the growth of chick oviducts [Abstract 2761]. Fed Proc 1975;34:705.) "Levonorgestrel can be fabricated into rods which are a homogeneous mixture of polydimethylsiloxane and crystalline levonorgestrel and covered with Silastic tubing." Here the one reference given (Robertson DN, Sivin I, Nash HA, Braun I, Dinh J. Contraception 1983;27:483) does not contain a description of rod fabrication.

A complete list of references of my work will be provided upon request.

Gopi N. Gupta, Ph.D.

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Reply to Gupta

To the Editors:

Dr. Dale Robertson of The Population Council, whose paper we cited for the description of the covered rods used in our study, advises me that Dr. Gupta does indeed deserve credit for devising some of the concepts used in the manufacture of the rods and in particular the use of polydimethylsiloxane without filler. These contributions were made while Dr. Gupta was a member of The Population Council staff. The rods we used differed from those described by Dr. Gupta in the methods used in their manufacture and in incorporating a cover consisting of Silastic tubing. The reference cited in our study was meant only to guide the interested reader to a description of the devices we used.

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Combined use of ritodrine and magnesium sulfate for tocolysis of preterm labor

To the Editors:

Ferguson et al. (Ferguson JE II, Hensleigh PH, Kredenster D. Adjunctive use of magnesium sulfate with ritodrine for preterm labor tocolysis. Am J Obstet Gynecol 1984;148:166) state that concomitant administration of ritodrine and magnesium sulfate for initiating tocolysis in patients with preterm labor was associated with a high rate (11 of 24) of cardiovascular complications. Because this high complication rate contrasted with our clinical impression concerning concomitant use of these agents, we reviewed our recent experience. Among 11 women receiving intravenous ritodrine and magnesium sulfate for premature labor, only one developed cardiovascular complications. This patient was a 26-year-old, gravida 2, para 1, white woman at 29 weeks' gestation with other risk factors (twin gestation and betamethasone treatment). She developed chest pain and dyspnea; evaluation revealed a normal electrocardiogram and a chest x-ray film consistent with pulmonary edema.

Explanation for the difference between these experiences is uncertain but may relate to differences in the manner in which magnesium sulfate was infused. In the protocol described by Ferguson et al., 13.2 gm of magnesium sulfate was administered during the first 2 hours, simultaneously with ritodrine. In contrast, we administered intravenous ritodrine alone unless contractions persisted despite maximum-dose ritodrine (0.35 mg/min) or there was maternal tachycardia (rates >120 bpm) or fetal tachycardia (rates >160 bpm). Under those conditions, magnesium sulfate was added (4 gm loading dose during 30 minutes followed by an infusion of 1 to 2 gm/hr), while the ritodrine infusion was decreased if necessary to eliminate maternal or fetal tachycardia.

The cardiovascular complications observed by Ferguson et al. may have resulted specifically from the effect of the large bolus of magnesium sulfate superimposed upon the inotropic and chronotropic effects of ritodrine. Support for this hypothesis are the previous observations that bolus infusion of magnesium sulfate is associated with a transient increase in cardiac output and cardiac index.[1, 2] Thus the effects of the combined agents may have increased the metabolic demands of the heart and exceeded the capacity for adequate oxygenation.

Because we included patients who either had failed ritodrine tocolysis or had tachycardia, we are unable to compare the efficacy of our protocol with Ferguson's protocol. Nevertheless, we were able to delay delivery a minimum of 2 days in five of 11 patients and for >10 days in three of these five.

While the explanation for the higher rate of cardiovascular complications in Ferguson's study is not clear, our clinical experience would suggest that we not yet preclude all treatment protocols of co-administration of magnesium sulfate and ritodrine. Further studies with different infusion protocols may be warranted to test both efficacy and safety.

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2. Mroczek WJ, Lee WR, Davidou ME. Effect of magnesium sulfate on cardiovascular hemodynamics. Angiology 1977; 10:720.

Reply to Diamond et al.

To the Editors:

The interest demonstrated by Diamond et al. in our study is appreciated. Clearly, the different methods of magnesium sulfate and ritodrine infusion and the prospective versus retrospective nature of the experiences do not allow a direct comparison. It is possible, but not probable, that the cardiovascular complications observed were exclusively the result of the bolus infusion of magnesium sulfate. As noted by Diamond et al., bolus infusions of magnesium sulfate have been associated with transient increases in cardiac output and cardiac index. However, in the study reported by Cotton et al. patients with severe preeclampsia were studied (perhaps not a good comparison group) and 4 gm of magnesium sulfate was infused during 15 minutes. Our patients received only 2.1 gm during each 15-minute interval in the first hour. Furthermore, 10 of the 11 symptomatic patients we described experienced adverse side effects at 6 hours or more after the initiation of magnesium sulfate therapy, a time when Cotton et al. demonstrated no effect on the cardiac index from an intravenous bolus infusion of magnesium sulfate.

We agree that future studies of different infusion methods (such as reported by Diamond et al.) may be warranted. We still believe, however, that "combination tocolytics *when used in the manner described herein* cause a significant increase in serious side effects and do not improve tocolytic efficacy."

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Transplacental spread of influenza virus

To the Editors:

The article by McGregor et al., which demonstrated the isolation of influenza virus in amniotic fluid (McGregor JA, Burns JC, Levin MJ, Burlington B, Meiklejohn G. Transplacental passage of influenza A/Bangkok (H_3N_2) mimicking amniotic fluid infection syndrome. Am J Obstet Gynecol 1984;149:856) is interesting. However, it is not the first such observation as was claimed. Yawn et al.[1] demonstrated transplacental transfer of influenza virus nearly 14 years ago, also by isolation from amniotic fluid.

The current article would be more correct if it claimed to be the first such infection with follow-up to term and with important data on fetal/neonatal immune response and observation of a healthy baby. It would be very important if the authors could tell us for how long after birth the virus was recoverable from the neonate. Can fetal infection with influenza virus result in chronic infection analogous to fetal rubella or cytomegalovirus infection?

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REFERENCE

1. Yawn DH, Pyeatte JC, Joseph M, Eichler SL, Garcia-Bunuel R. Transplacental transfer of influenza virus. JAMA 1971;216:1022-3.

Reply to Amstey

To the Editors:

We are thankful for Dr. Amstey's comments. He is an acknowledged leader in the study of maternal and perinatal infections. We agree that our paper deserves careful reading. We stated that this was the first "confirmation of transplacental influenza infection." We discussed and referred to Yawn et al.'s report of virologic recovery of influenza virus from amniotic fluid at necropsy in a pregnant woman who rapidly died of overwhelming influenza virus (A2/Hong Kong/8/68) prominently in the first and ninth paragraphs of our article. Microbiologic recovery of organisms from body tissues and fluids at autopsy does not necessarily prove ongoing infection. Yawn et al. found no histologic or serologic evidence of fetal infection. Further, we discussed and detailed knowledge of extrapulmonary spread of influenza virus in pregnant and nonpregnant individuals. We concluded: "It is unclear why there has only been a single previously reported passage of influenza virus from the human mother to her fetus," referring to Yawn's observations. We observed maternal/fetal transmission of influenza virus with documentation of fetal infection with specific IgM and IgA serologic testing. Few other observations of extrapulmonary spread of influenza virus in a human are as well documented. Also, we emphasize that maternal influenza infection may be clinically mistaken as amniotic fluid infection, thus bringing about untimely iatrogenc delivery.

Dr. Amstey's second question regarding the recovery of virus in the newborn infant is of great importance. We stated in the "virologic evaluation" section of our article: "At delivery, viruses could not be isolated from the baby's nasal washings or from the placenta or amniotic fluid." These data are presented in Table IA. The child and mother were followed up for 3 months after delivery. No sequelae were noted in the mother or newborn infant. We did not find evidence of any chronic infection.

Thank you for allowing us to clarify and emphasize these points.

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Tubal ligation: A misnomer

To the Editors:

"Once upon a time," in the year 1881, in Toledo, Ohio, a physician named S. S. Langren[1] described the procedure of tubal ligation. He performed the operation post partum on a patient after her second cesarean section. In his paper he stated that he "first intended to remove the ovaries with Smith's pile clamps and cautery, but in consideration of the danger of hemorrhage jeopardizing the operation, the fallopian tubes were tied instead with a strong silk ligature about one inch from the uterine attachment." Hence the term "tubal ligation" has prevailed and flourished through the decades for more than 100 years. It would be difficult if not impossible, to know when this procedure was abandoned for more specific and effective techniques. In the South Bronx, even today, we still have patients requesting that their "tubes be tied" and "not cut." The request is made with the concept that they may be "untied" at a later date, when they are better prepared for further pregnancy. The permanent status of infertility suggested by "cutting" the tubes as opposed to "tying" them is apparently emotionally unacceptable. According to several of my colleagues from the Caribbean, India, and Africa, the procedure is still being performed in certain regions. There are also reports of subsequent pregnancies occurring after tubal ligation. This apparently represents either recanalization of the fallopian tubes or failure to ligate them properly.

Table I. Currently used methods of tubal sterilization

- Abdominal
  - Laparotomy
  - Minilaparotomy
    - Pomeroy
    - Irving
    - Madlener (noncutting)
  - Salpingectomy
    - Total or partial
- Vaginal approach for above
- Laparoscopic
  - Electrocautery
  - Falope ring
  - Clips
    - Hulka
    - Filshie
- Hysteroscopic
  - Silicone plugs
  - Tubal sclerosing agents

It is interesting that medical and all third-party insurance carriers still accept and honor the term "tubal ligation," although medicaid codes it more specifically and appropriately as "tubal transection."

The current most used and acceptable methods for tubal interruption are listed in Table I.

With the current emphasis on quality assurance, meaningful informed consent, and proper documentation, the term "tubal ligation" should be abandoned. In its place the term "tubal transection" is more appropriate, or the term "tubal interruption" with the specific procedure used.

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REFERENCE

1. Langren SS. A case of cesarean section twice successfully performed on the same patient. Am J Obstet Dis Women 1881;14:78-94.

Sound stimulation test and fetal well-being

To the Editors:

An article by Serafini et al. (Serafini P, Lindsay MBJ, Nagey DA, Pupkin MJ, Tseng P, Crenshaw C Jr. Antepartum fetal heart rate response to sound stimulation: the acoustic stimulation test. AM J OBSTET GYNECOL, 1984;148:41-51) suggested that an acoustic stimulation test is able to replace the traditional nonstress test (NST) in the screening of hypoxic fetuses. Another question is the value of this test as a complementary procedure in case of a nonreactive NST. A recent work by our group[1] indicates that a sound stimulation test could be useful in this case. We used a 5-second, 110 dB white noise generated by a loudspeaker. Unlike Serafini et al., we submitted the mother to a masking noise delivered by a headphone, in order to avoid indirect fetal stimulation through maternal reactions. A total of 93 tests were performed in 78 high-risk pregnancies with suspicious or abnormal NST recordings (Fischer's score ≤7). The results showed a good correlation between the NST and the sound stimulation test. The sound stimulation test was negative (no fetal response) in 10%, 48%, and 100%, respectively, of NSTs with Fischer's score of 7, 6, or ≤5. Thus we have confirmed that a poor fetal reaction to acoustic stimuli is correlated with the severity of hypoxia. When the sound stimulation test is not used as a primary screening test, baseline NST patterns should be taken into account. In case of severe abnormalities of the NST, no complementary test is needed (100% of 11 fetuses with Fischer's score ≤5 showed a negative sound stimulation test and low Apgar score and/or acidosis at birth).

On the other hand, when the NST is only suspicious (Fischer's score of 6 or 7), the sound stimulation test has a good discriminatory value in detecting the risk of neonatal distress. Among 62 such cases evaluated within 7 days of delivery, a 27% (17 of 62) overall rate of neonatal depression (low Apgar score at 5 minutes and/or acidosis in the umbilical artery at birth) was noticed. The sound stimulation test was interpreted as reactive in 44 cases of this group, with only eight depressed neonates (18%). In the other 18 cases, a negative, nonreactive sound stimulation test was followed by birth of nine depressed infants (50%). The difference is statistically significant ($p < 0.02$). Thus we have confirmed that a sound stimulation test is a low-cost, easy, and reliable procedure, useful in the prognostic evaluation of a suspicious NST.

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Gestational age according to fetal sex in twins

To the Editors:

Inspired by the twin study on duration of gestation according to fetal sex published by Newton et al. (Newton W, Keith L, Keith D. The Northwestern University multihospital twin study. IV. Duration of gestation according to fetal sex. AM J OBSTET GYNECOL 1984;149:655) we reconsidered our twin data published in 1979.[1]

We studied 1655 twin pairs with a menstrual age >16 weeks born at the Department of Obstetrics and Gynecology of the University Hospital between 1931 and 1975 and at the training school for midwives from 1948 to 1957, both in Amsterdam. In 1485 twin pairs, gestational age, placentation, parity, and fetal sex were known. Statistical procedures used were multiple regression analyses and analyses of variance.

In Table I we present the mean gestational age of 1485 twins according to placentation, parity, and sex; monochorial twins were found to have a significantly shorter duration of pregnancy than dichorial twins by 10.6 days. The mean gestational age of twin pregnancies in multiparous women was found to be significantly longer than in primiparous women by 3.9 days. As is obvious from Table I and in common with Newton et al., male twin pregnancies were found to be shorter than both female and male/female twins; however, this difference was not statistically significant at the 0.05 level.

However, we cannot confirm the findings concerning birth weights in twins given by Newton et al. Therefore, Table I also contains the mean birth weight according to placentation, parity, and sex. Dichorial twin infants were found to be heavier than monochorial twin infants. In complete accordance with observations in singleton infants[2] we found that twin infants of multiparous women were heavier than those of primiparous women (mean effect of parity: 132 gm) and that, in twins, boys were heavier than girls (mean effect of sex: 121 gm).[1] All of these differences were statistically significant.

Obviously male twin pregnancies show a somewhat shorter mean duration of gestational age, although not statistically significant, as compared to that of other twins.

Table I. Mean gestational age and mean birth weight in 1485 twin pairs in relation to placentation, parity, and fetal sex

Twin pairs	*Sex*	*No. of twin pairs*	*Mean gestational age (days)*	*Mean birth weight (gm)*
Monochorial (n = 349)				
Primiparous women	Male-male	81	246.5	2030
	Female-female	73	243.9	1957
Multiparous women	Male-male	94	247.4	2284
	Female-female	101	251.4	2179
Dichorial (n = 1136)				
Primiparous women	Male-male	151	255.8	2289
	Female-female	133	256.2	2234
	Male-female	154	256.4	2346
Multiparous women	Male-male	201	258.4	2518
	Female-female	189	261.3	2463
	Male-female	308	260.5	2534

One explanation could be that male twins, being heavier, are associated with a more distended uterus. Twins as compared to singleton infants[2] and cases of polyhydramnios[1] clearly demonstrate a shorter duration of gestational age related to distention of the uterus. Since, as in singleton infants,[2] placental weights of male and female children were found to be the same,[1] another explanation could be the relatively smaller amount of placental tissue (or lower placental index) available to those male twins, possible related to an earlier start of labor.

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1. Bleker OP, Breur W, Huidekoper BL. A study of birth weight, placental weight and mortality of twins as compared to singletons. Br J Obstet Gynaecol 1979;86:111.
2. Kloosterman G. On intrauterine growth: the significance of prenatal care. Int J Gynaecol Obstet 1970;8:895.

Reply to Bleker and Hemrika

To the Editors:

In general, analyses of twin gestation with respect to sex have tended to lump together all infants of one sex and compare them with all infants of the other sex, regardless of the sex combination of the individual pregnancies. Our article addressed this seemingly oversimple approach.

In this context, the reanalysis by Bleker and Hemrika of their previously published data is of great interest. We are encouraged by their agreement with our findings that twin male gestations were shorter than other twin gestations, as measured by dates. However, with respect to birth weight, although we appreciate the size of their sample, we believe that the disagreement between our findings and theirs must be resolved at the bar of evidence from other twin series. This is particularly true since their sample group differs substantially from ours in racial configuration as well as the fact that the cases were collected over 34 years.

Our most significant finding, the difference in gestational age based on pediatric examination, has no counterpart in the data presented by Bleker and Hemrika. In our opinion, this finding is important, for it suggests that, in the opinion of the physician at the scene, male twins were significantly less mature than other twins in terms of behavioral and morphologic traits.

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ITEMS

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Adolescent Pregnancy: Problems & Solutions, May 17, 1985, The Palm Beach Airport Hilton International Ballroom, 150 Australian Ave., West Palm Beach, FL 33402. For more information contact: Becky Myers, Planned Parenthood, 5312 Broadway, West Palm Beach, FL 33407. Tel.: (305) 848-6300.

18th Annual Postgraduate Course in Gynecologic Pathology, Endocrinology and Maternal-Fetal Medicine, June 3-8, 1985, Olympia Resort and Spa, Oconomowoc, Wisconsin. For further details contact: Richard F. Mattingly, M.D., Medical College of Wisconsin, 8700 West Wisconsin Ave., Milwaukee, WI 53226.

Basic & Advanced Colposcopy/Laser Seminar, June 27-29, 1985, Grand Hyatt Hotel, New York, New York. For further information contact: Dr. Ralph Richart, 630 West 168th St., New York, New York. Tel.: (212) 694-3623.

Issues in Maternal-Fetal Medicine: Teratology, Principles and Clinical Applications, July 27–August 1, 1985, Snowmass, Colorado. For further information contact: CME Office, Texas A&M University College of Medicine, Scott and White, Temple TX 76508. Tel.: (817) 774-2350.

Practical Management of Infertility, March 29-30, 1985, Ramada Hotel Ballroom, Tyler, Texas. For additional information call: Mr. Howard Kaplan, Serono Symposia, U.S.A. Tel.: (800) 225-5185.

Tutorial Workshops for the In Vitro Fertilization of Human Oocytes, April 29–May 3, 1985. For additional information contact: In Vitro Program Coordinator, Mt. Sinai Medical Center, One Mt. Sinai Dr., Cleveland, OH 44106. Tel.: (216) 421-5884.

Advanced Gynecologic Microsurgical Technique, June 21-22, 1985, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut. For further information contact: Office of Graduate and Continuing Education, Yale University School of Medicine, 333 Cedar St., P. O. Box 3333, New Haven, CT 06510. Tel.: (203) 785-4578.

Fourth Annual Fetal Surgery Meeting and Workshop, June 9-11, 1985, Jasper Park Lodge, Jasper, Alberta, Canada. For further information contact: Dr. F. A. Manning, President, International Fetal Surgery Registry, WS-123 Women's Hospital, 735 Notre Dame Ave., Winnipeg, Manitoba, Canada R3E 0L8.

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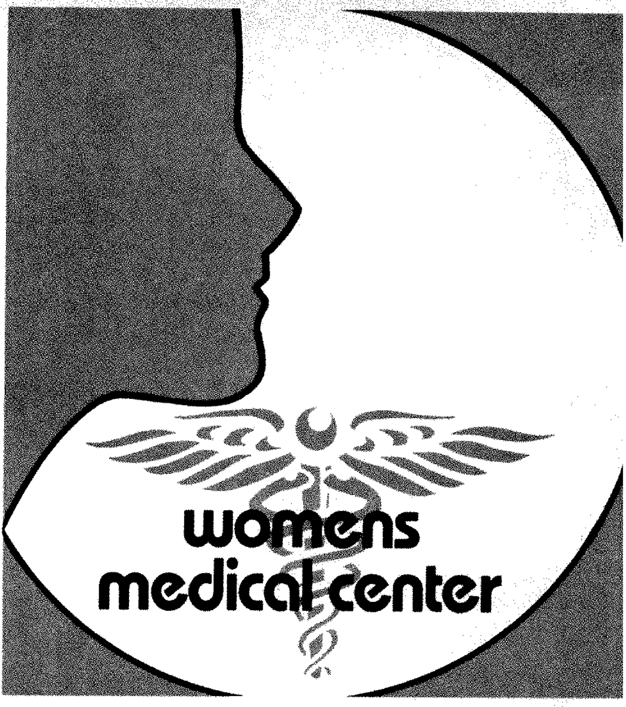

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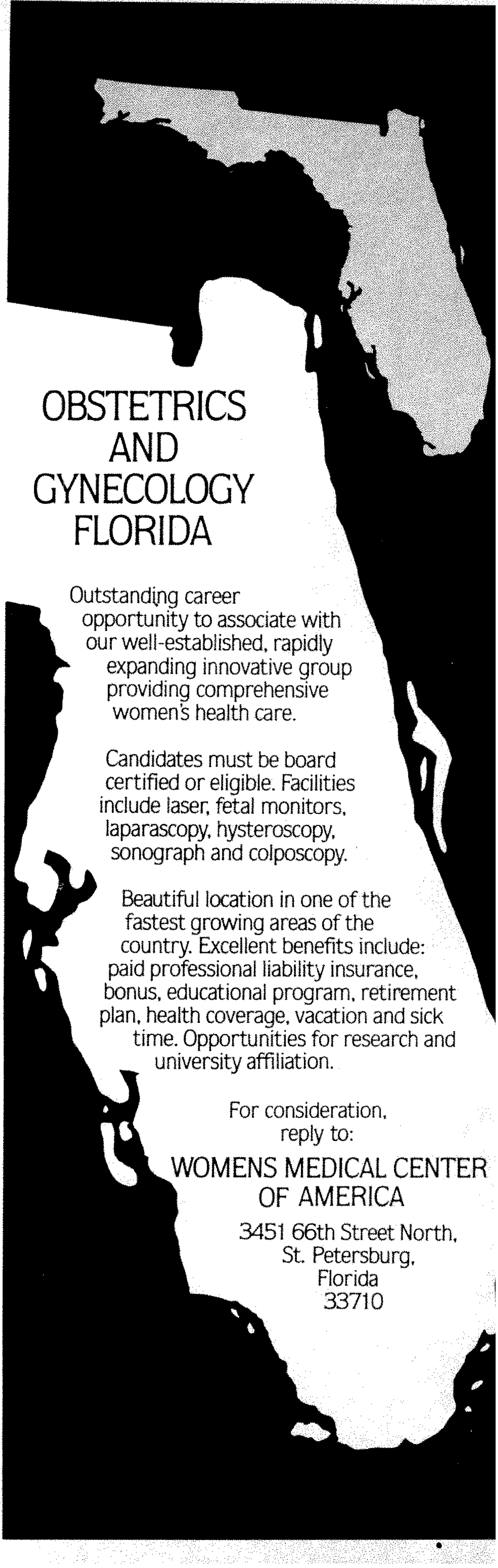

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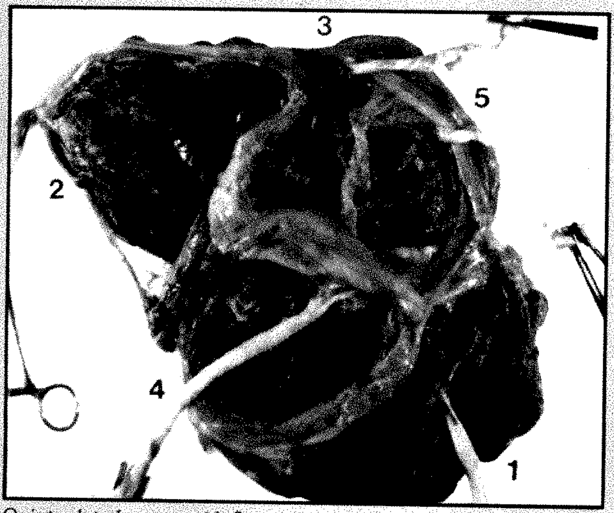

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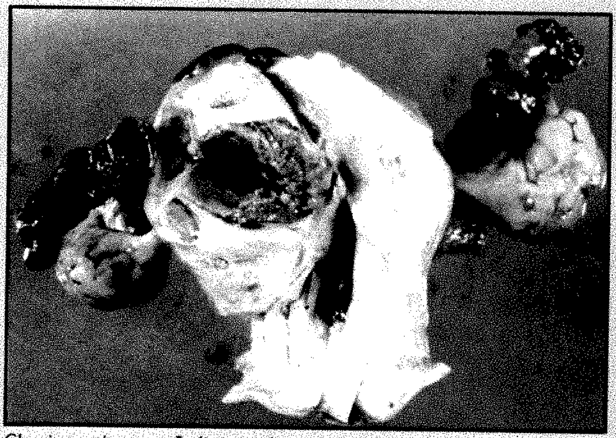

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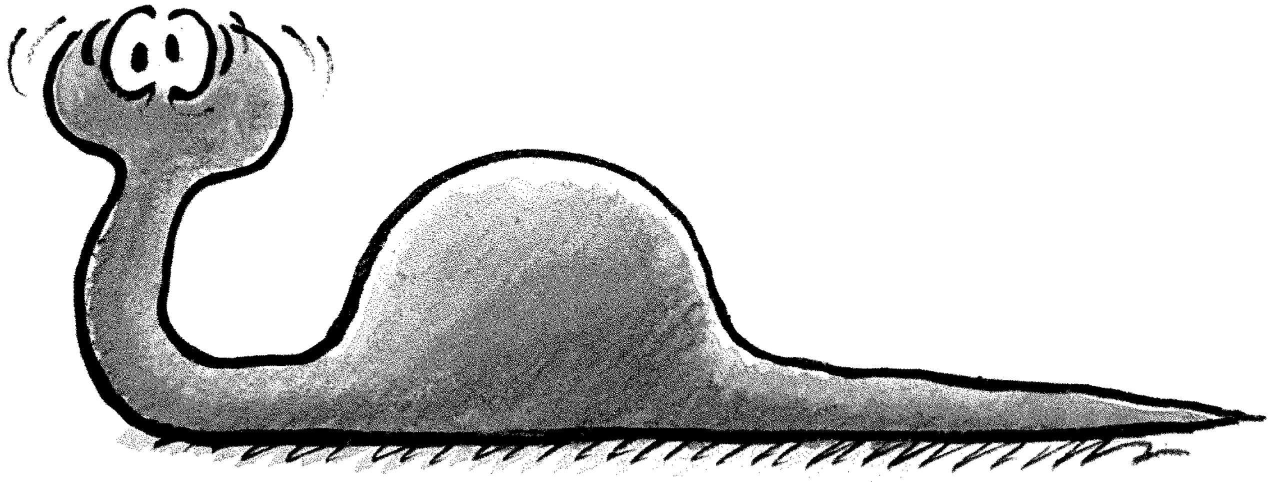

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Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING
CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years

Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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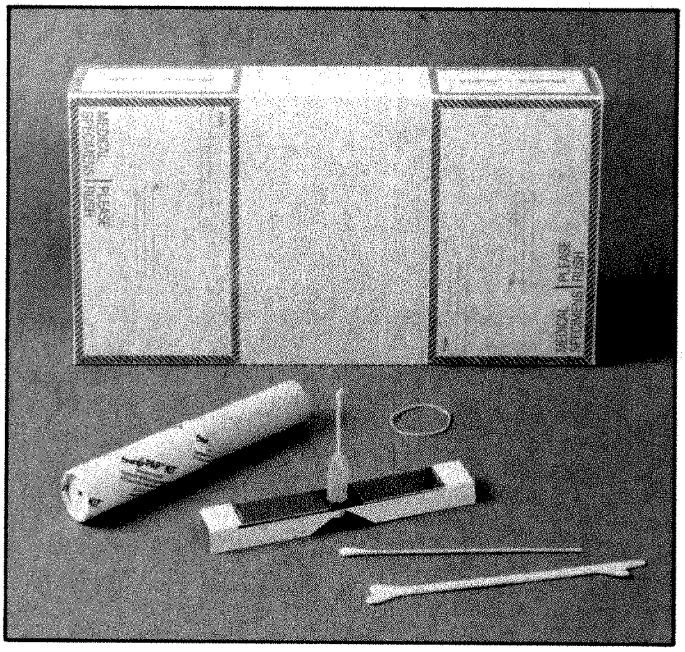

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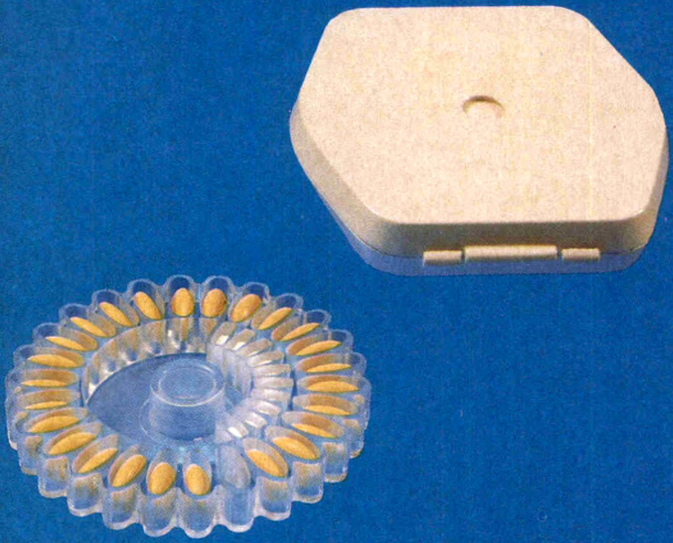

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Indications: Pod-Ben-25 is to be used for the removal of warts (including condylomas).

Cautions: **Pod-Ben-25 is an extremely potent medicinal and is to be used (applied) only by the doctor.** Do not use if wart or surrounding tissue is inflamed or irritated. Care and caution should be used in selection of patients to be treated with Pod-Ben-25. Do not use for pregnant women, diabetics, or people using steroids or with poor blood-circulation; nor on bleeding warts, moles, birthmarks, or unusual warts with hair growing from them; nor should more than 0.5 ml be applied at one time.(4) It is recommended that patient be advised of effect and possible results of treatment. Keep away from the eyes; if any contacts eye flush with warm water thoroughly and treat as condition indicates.

A noteworthy summary of the position and problem of podophyllin therapy was given by Dr. A. A. Fisher in the opening paragraphs of his September 1981 article in Cutis:

"Podophyllum resin remains the treatment of choice for condylomata acuminata (venereal warts). As a rule, an intense inflammatory reaction occurs which may become quite painful for a few days. Usually the treatment is successful and there are no local or systemic sequelae. Occasionally, however, severe systemic and local reactions do occur.

. . . . Although many dermatologists have used topical podophyllum resin for many years without encountering systemic reactions in their patients, a review of the literature reveals several reports of severe systemic reactions. Among the various systemic effects that have been reported from the use of topically applied podophyllum resin are the following: paresthesia, polyneuritis, paralytic ileus, pyrexia leukopenia, thrombocytopenia, coma, and death." (3)

Method: Pod-Ben-25 is applied to cleansed lesion, using appropriate applicator and allowed to dry thoroughly. Only intact (no bleeding) lesions should be treated and contact with healthy tissue is to be avoided. Due to possible extra-sensitivity to the drug it is recommended that the lotion be first left in contact for a short time (30 to 40 minutes) to determine patient's reaction, if any. To avoid systemic absorption, time of contact should be minimum time necessary to produce the desired result (1 to 4 hours, depending on condition of lesion and of patient), the doctor developing his own experience and technique. Large areas or numerous warts should not be treated at once.

The dried lotion can be removed by alcohol or soap and water; removal should be complete.

Note: **Pod-Ben-25 is available from C&M ONLY by direct shipment to doctor's office.**

References:

(1) Bettley: The treatment of skin carcinoma with podophyllum derivatives; Brit. J. Dermat., **84**, 74 (1971)

(2) Zackheim: Hazards of topical mitotic-blocking agents; Arch. Dermat. **113**, 234 (1977)

(3) Fisher: Severe systemic and local reactions to topical podophyllum resin; Cutis, **28**, 233 (1981)*

(4) Krogh: Condylomata Acuminata; Sem. in Dermat., **2**, 109 (1983)

**An extensive list of references to podophyllin in the literature is provided at the end of Dr. Fisher's article.*

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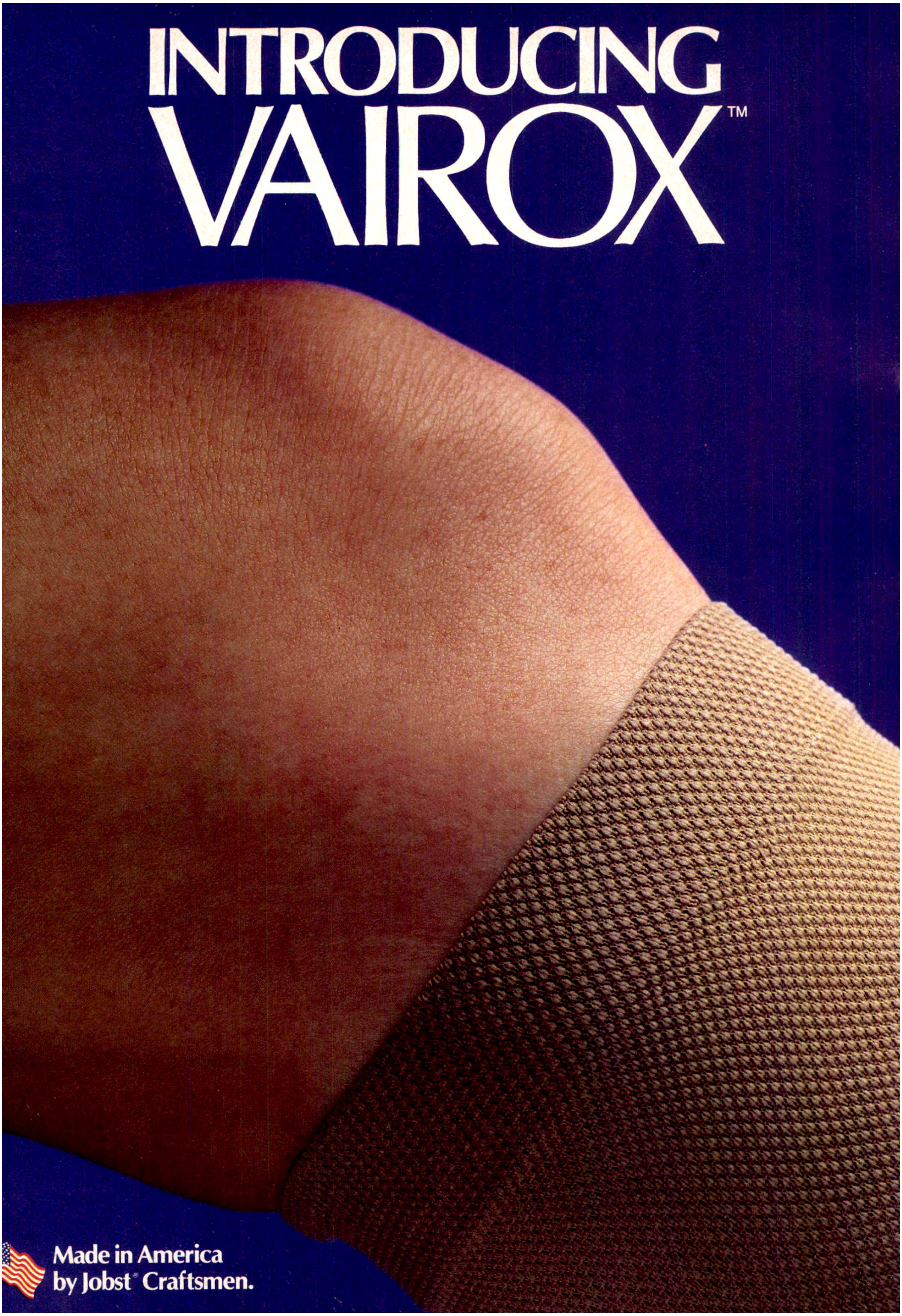
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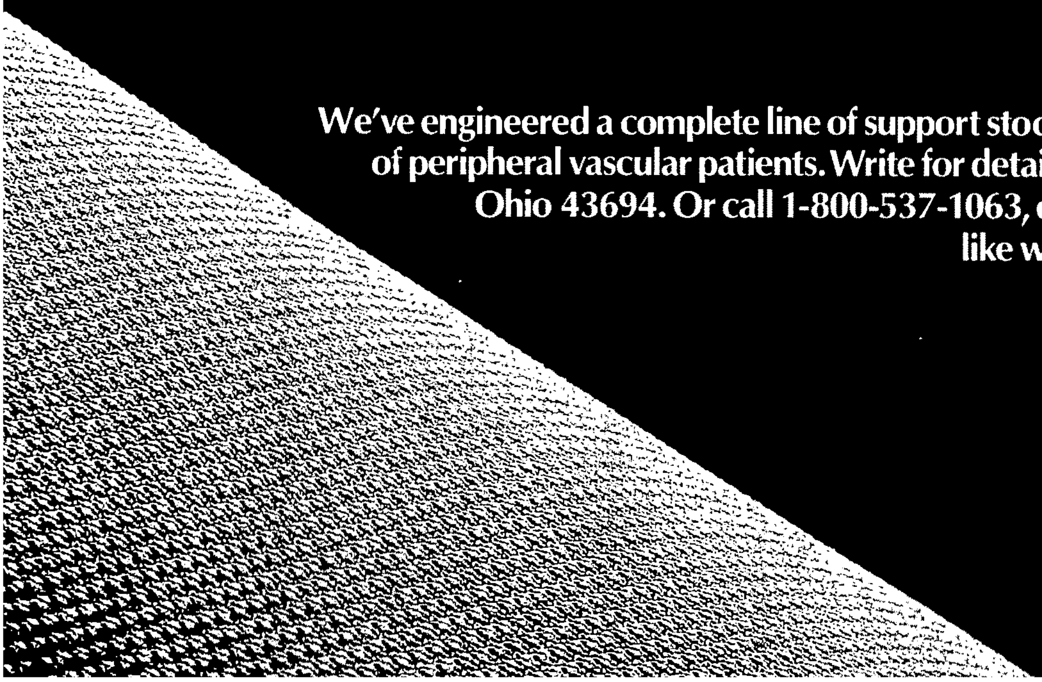

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NEW!

MEDICAL DISORDERS IN OBSTETRIC PRACTICE

Edited by Michael de Swiet, M.D., F.R.C.P.; with 9 contributors.

"The fact that two lives are at risk, and the varying physiology during the course of pregnancy and post partum make the bravest of us need a source of help and instruction. The book edited by Michael de Swiet fulfills this role admirably . . . the book as a whole is excellent and highly recommended. It is a book to buy." (The Lancet)

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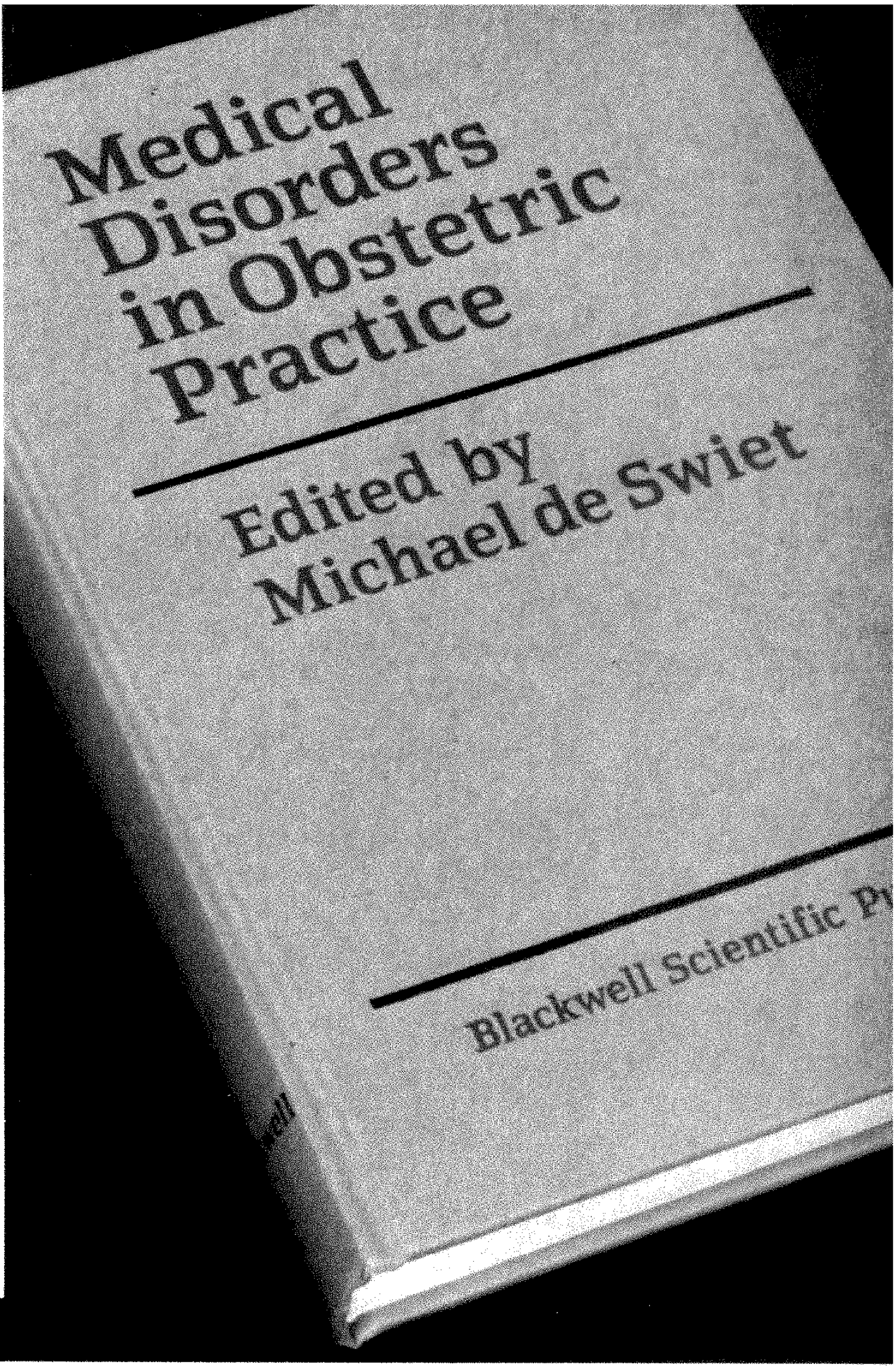
1984. 534 pages, 53 illustrations. (B-1263-2) Mosby distributes this Blackwell Scientific Publication. $58.95.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Number of women with and without withdrawal bleeding, taking either OGEN 1.25 or conjugated estrogens 0.625 mg

	No Withdrawal Bleeding
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Adapted from Wren et al.[1]

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(estropipate)

Only estrone. Identical to the body's own.

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*Both regimens were given 24 days out of 30, with levonorgestrel 0.03 mg added days 15 to 24.

Scored tablets permit more precise dosage

OGEN .625
(estropipate 0.75 mg, calculated as sodium estrone sulfate 0.625 mg)

OGEN 1.25
(estropipate 1.5 mg, calculated as sodium estrone sulfate 1.25 mg)

OGEN 2.5
(estropipate 3 mg, calculated as sodium estrone sulfate 2.5 mg)

OGEN 5
(estropipate 6 mg, calculated as sodium estrone sulfate 5 mg)

■ *Unlike conjugated estrogen tablets, scored OGEN tablets permit the use of half tablets. So you can adjust precisely to the minimum needed for symptom control. One more reason more doctors are considering OGEN. And more women are glad they are.*

See adjacent page for brief summary.

Reference:
1. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

Abbott Pharmaceuticals, Inc
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OGEN®

ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods.[1-3] This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.[4]

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment[1] and on estrogen dose.[3] In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration;[3] it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.[5,6] In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures.[7] Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis,[8-12] epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects.[13-16] One case control study[16] estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS

OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. Known or suspected pregnancy (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,[17] although a recent long-term followup of a single physician's practice has raised this possibility.[18] Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens,[17] similar to the 2-fold increase previously noted in users of oral contraceptives.[19,22] In the case of oral contraceptives, the increased risk appeared after two years of use.[22]

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.[20,21]

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.[22-29] Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug.[30,31] An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives.[32,33] If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found[17,34] this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men[35] to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives.[36-38] Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives.[37] The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause[39] and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression.[22] Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

10. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

11. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. OGEN (estropipate tablets) cannott be recommended for use during pregnancy. See Contraindications and Boxed Warning.

D. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Breakthrough bleeding, spotting, change in menstrual flow.
Dysmenorrhea.
Premenstrual-like syndrome.
Amenorrhea during and after treatment.
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Change in cervical eversion and in degree of cervical secretion.
Cystitis-like syndrome.

2. *Breast.*
Tenderness, enlargement, secretion.

3. *Gastrointestinal.*
Nausea, vomiting.
Abdominal cramps, bloating.
Cholestatic jaundice.

4. *Skin.*
Chloasma or melasma which may persist when drug is discontinued.
Erythema multiforme.
Erythema nodosum.
Hemorrhagic eruption.
Loss of scalp hair.
Hirsutism.

5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.

6. *CNS.*
Headache, migraine, dizziness.
Mental depression.
Chorea.

7. *Miscellaneous.*
Increase or decrease in weight.
Reduced carbohydrate tolerance.
Aggravation of porphyria.
Edema.
Changes in libido.

ACUTE OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

The usual dosage range is one OGEN .625 (estropipate) Tablet to one OGEN 5 Tablet per day.

2. *Given cyclically:*

Female hypogonadism. Female castration. Primary ovarian failure. The lowest dose that will control symptoms should be chosen. A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The same schedule is repeated if bleeding does not occur by the end of the rest period. The duration of cyclic estrogen therapy necessary to produce withdrawal bleeding will vary according to the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

5043716

Abbott Laboratories
Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064

Mycelex®-G:
The original 3-night therapy for vulvovaginal candidiasis

Tried...

☐ Efficacy supported by more than 10 years of worldwide clinical experience.

☐ More than 200,000 new prescriptions in 1983 in the U.S. alone.[1]

Trusted...

☐ Clinical* and mycologic cure† after 3 nights of treatment.‡

☐ Low relapse or reinfection rate one month after treatment.

☐ Low (1.6%) incidence of side effects.[1]

Easy-to-use...

☐ Easy to handle *non-waxy* tablets disperse quickly without the mess and leakage of suppository "meltables."

☐ Non-waxy tablets are simple to position with applicator or by hand.

☐ Compliance is encouraged by the simple-to-follow 3-night regimen and easy-to-remember "before bedtime" use.

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309310
Mycelex®-G 100mg
(clotrimazole) Vaginal Tablets
7 Vaginal Tablets and applicator
Caution: Federal law prohibits dispensing without prescription.
MILES

Mycelex®-G

(clotrimazole) 100 mg vaginal tablets

MILES
Miles Pharmaceuticals

Reference:
1. Data on file, Miles Pharamaceuticals.

*Absence of signs and symptoms.

†Negative cultures.

‡For pregnant patients, the 7-day course of therapy must be used.

Please see next page for Brief Summary of prescribing information.

Miles Pharmaceuticals
Division of Miles Laboratories, Inc.
West Haven, Connecticut 06516 USA

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Patient accepted, non-waxy, 3-night therapy

Mycelex-G®

(clotrimazole) 100 mg vaginal tablets

ACTIONS: Clotrimazole is a broad-spectrum antifungal agent that inhibits the growth of pathogenic yeasts. Clotrimazole exhibits fungicidal activity *in vitro* against *Candida albicans* and other species of the genus *Candida*.

No single-step or multiple-step resistance to clotrimazole has developed during successive passages of *Candida albicans*.

INDICATIONS: Mycelex-G Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis *(Trichomonas* and *Hemophilus vaginalis)* should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex-G Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

PRECAUTIONS: **Laboratory Tests:** If there is a lack of response to Mycelex-G Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

Application of ^{14}C-labeled clotrimazole has shown negligible absorption (peak of 0.03 mcg/ml of serum 24 hours after insertion of a 100 mg tablet) from both normal and inflamed human vaginal mucosa.

Usage in Pregnancy: While Mycelex-G Vaginal Tablets have not been studied in the first trimester of pregnancy, use in the second and third trimesters has not been associated with ill effects. Follow-up reports now available on 71 neonates of 117 pregnant patients reveal no adverse effects or complications attributable to Mycelex-G therapy.

ADVERSE REACTIONS: Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints, such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet a day for seven consecutive days. Recent studies indicate that an alternative regimen of two tablets a day for three consecutive days is similarly effective in nonpregnant patients; however, in pregnant patients the three-day treatment course did not prove to be effective and is therefore not recommended. They should receive the seven-day treatment. Using the applicator supplied, insert one or two tablets intravaginally preferably at bedtime.

In the event of treatment failure, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy. There are no studies to show whether a second course of clotrimazole would be effective in patients who fail to respond to the initial course.

HOW SUPPLIED: Mycelex-G Vaginal Tablets 100 mg, white, tear-drop-shaped, uncoated tablets, supplied in a strip of seven tablets with plastic applicator and patient leaflet of instructions.

Do not store above 35°C (95°F).

DPSC Stocked: NSN 6505-01-090-6795

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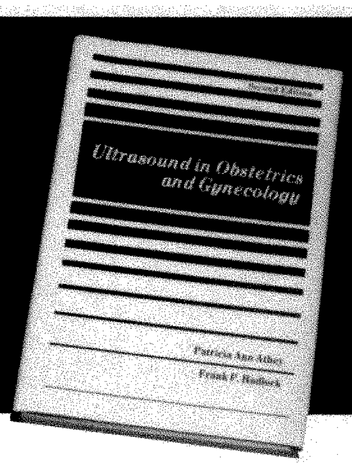

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By Patricia Ann Athey, M.D. and Frank P. Hadlock, M.D.; with 11 contributors.

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- vaginal abnormalities
- uterus: abnormalities of the endometrial cavity
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- pelvic inflammatory disease
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INFORMATION FOR AUTHORS

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References:
1. Rössner S et al: *Acta Obstet Gynecol Scand* 59:255, 1980. 2. Nash AL et al: *Med J Aust* 2:277, 1979. 3. Larsson-Cohn U et al: *Fertil Steril* 35:172, 1981. 4. Briggs MH: *J Reprod Med* 28:92, 1983. 5. Åhrén T et al: *Contraception* 24:451, 1981. 6. Briggs MH, Briggs M: *Acta Obstet Gynecol Scand* Suppl 105:25, 1982.

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Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulato or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encourag to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in th milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressur breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients wi conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaire liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible tha if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components ma be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention.
b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltere
d. Decreased pregnanediol excretion.
e. Reduced response to metyrapone test

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reaction has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vagina candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NORDETTE* each tablet contains
0.15 mg levonorgestrel and
0.03 mg ethinyl estradiol.

TABLETS

(LEVONORGESTREL AND ETHINYL ESTRADIOL TABLETS)

Wyeth Laboratories
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TRANSACTIONS OF THE THIRD ANNUAL MEETING OF THE AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY

It's what you learn after you know it all that really counts*

Presidential address

E. J. Quilligan, M.D., and Howard L. Stone, Ph.D.
Madison, Wisconsin

There are at least two times in a physician's life that possession of all knowledge seems to be a certainty. The first is upon graduation from medical school. Having gone through the agony of the basic science years in which the foundation for a lifetime in medicine is imprinted and then taking this knowledge to patients' bedsides, where success in resolving patients' problems can be achieved under the tutelage of the best instructors, gives one a feeling of invincibility. This feeling lasts until about the first day of the internship when the reality that you are suddenly on your own becomes apparent. You realize that there is much more to medicine than you were taught in medical school. The second period in which physicians become reasonably certain that they know it all is in the latter portion of their chief residency. They have, after all, worked with some of the best people in the country and, furthermore, have managed patients alone. This feeling usually lasts until their first difficult case in private practice and then suddenly they realize they were not alone in their residency, but they are now.

I doubt that there is really any day in a medical career when deep in their soul physicians truly believe that they know it all. If there are such persons, and there might be a few, they are a great danger to their patients Medicine is not only a jealous mistress, requiring much of one's time, but also a jealous school mistress, requiring that one learn all the time. All of our state and national organizations have recognized this need for continuing education. Thirty-five states require a certain number of hours of postgraduate education to keep one's license current.[1] The American Board of Obstetrics and Gynecology has strongly urged voluntary recertification and in the future will give a time-limited certificate that will force retesting the individual after 10 years in practice.

Never before in the field of medicine have we had such a varied menu of postgraduate entrées. They include journals, books, postgraduate courses, grand rounds, and untold numbers of hospital conferences.

A brief survey of the cumulative index reveals that there are 31 journals that are written in English and devote their pages exclusively to the topic of obstetrics and gynecology. In the year from June, 1983, to June, 1984, over 4000 articles were published in these journals; however, 4000 is an underestimate of what one would have to read to truly keep up with the literature because many excellent obstetric and gynecologic articles are written in nonspecialty journals such as *The New England Journal of Medicine, The Journal of the American Medical Association*, and many others. To be truly abreast of the literature, an obstetrician/gynecologist would have to read more than 10 articles per day, 365 days per year. I do not wish to intimate that all 4000-plus articles are worth reading. Many of them are not; they are simply another article that someone has written to achieve tenure rather than to enlighten the reading public. However, the number of articles cluttering the literature for purposes other than scientific advancement is not the subject of this talk, so I will simply say that it poses a dilemma of choice for the reading ob-

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From the Departments of Obstetrics and Gynecology and Educational Resources, University of Wisconsin.

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**From a book of the same title by Earl Weaver.*

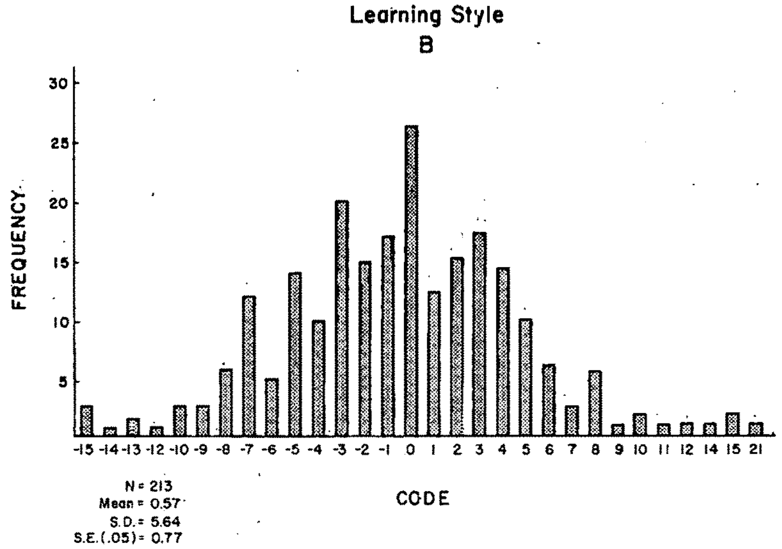

Fig. 1. Preferred learning style index applied to the best group *(B)*. A positive score represents a discovery learner and a negative score represents a receptive learner.

Table I. Practice specialty

	Best group		Average group	
	n	%	*n*	%
Obstetrics/ Gynecology	197	86	174	87
Obstetrics	3	1	2	1
Gynecology	29	13	22	11
Total	229		200	

stetrician/gynecologist. A part of the dilemma is self-solving because some journals arrive free on one's doorstep, such as *Contemporary Ob/Gyn* and the *Journal of Reproductive Medicine*, and others such as *Obstetrics and Gynecology* are received because of membership in the American College of Obstetricians and Gynecologists.

The absolute explosion in the number of obstetric and gynecologic postgraduate courses during the past 10 years is undoubtably a tribute to the desire for medical education and the current income tax structure. One can go to Europe to learn the finer aspects of delivering a baby in the United States or go on a shipboard cruise to learn the latest in the treatment of infertility. There are those who would argue that it might be better to send the infertile couple on the cruise and have the physician stay at home. Out of interest I have saved all of the postgraduate brochures that I have received in the mail over the past year, which certainly does not include all of the postgraduate courses given during the year. There were 68 brochures received in my office. The meetings lasted from 1 to 16 days, with from 6 to 44 hours of instruction involving from 2 to 87 instructors. The average meeting lasted 3 days and utilized 13 instructors. This list did not include the American College of Obstetricians and Gynecologists' annual or sectional meetings or any of the regional society meetings such as those of the Pacific Coast Obstetrical and Gynecological Society, the Central Association of Obstetricians and Gynecologists, and the South Atlantic Association of Obstetricians and Gynecologists. It was impossible for me to find an absolutely complete listing of all the postgraduate courses in our specialty. The best I could find was in the *Ob/Gyn News*, which listed 86 courses over the period of August to December, 1984. If this sample is representative, roughly 200 courses are given each year. We can assume from the general figures given above that the 200 courses would utilize 2600 instructors for 3 days. Since there are about 1600 full-time physician faculty members in medical schools,[2] each would be teaching one to two postgraduate courses per year. This would seem to be a modest commitment; however, the major burden of teaching these postgraduate courses falls on a

Table II. Type of practice

	Best group		Average group	
	n	%	*n*	%
Solo*	62	27	87	43
Group†	121	53	56	28
Health maintenance organization	1	0	3	1
Partnership	38	17	44	22
Other	7	3	10	5

*$p < 0.001$.
†$p < 0.001$.

Table III. Type of school of medicine

	Best group		*Average group*	
	n	%	*n*	%
State*	99	49	135	59
Private	100	50	92	40
Other	1		2	

*p < 0.001.

Table IV. Academic standing

	Best group		*Average group*	
Quadrant	*n*	%	*n*	%
Not given	9	4	12	6
Upper*	113	49	74	37
Upper middle	84	37	83	41
Lower middle	20	9	28	14
Lower	3	1	3	1

*p < 0.001.

Table V. Location of residency

	Best group		*Average group*	
	n	%	*n*	%
Private*	46	20	58	29
University†	130	57	95	47
County	37	16	25	13
Federal	7	3	4	1
Other	9	4	21	10

*p < 0.001.
†p < 0.01.

small proportion of this total faculty, creating significant periods of travel time for those actively engaged in postgraduate education.

Without going into the number of new textbooks written each year or the number of local conferences such as grand rounds and perinatal conferences, it seems apparent that we have a massive educational effort being put forth in the United States. It is expensive in time and money. Miller[3] estimated in 1977 that the cost of continuing medical education in the United States was almost $2 billion if one includes lost physician time. That figure has probably increased substantially by 1984. Is it being utilized efficiently, who is using it, and does it really lead to better patient care?

We have attempted to answer some of the above questions by using a different approach, a survey of what some people in practice are actually doing. The survey was constructed and sent to all of the chairmen of obstetrics and gynecology departments in the United States. The instructions to the chairmen were to pick the five best practitioners in the community and five average practitioners and send to each a precoded survey. The survey included general data, such as the medical school from which the physician graduated, year of graduation, rank in the class, and specific questions designed to determine how physicians obtained their postgraduate education and how this correlated with their learning style. The learning style was assessed by use of a questionnaire that had been developed by the Division of Education of the University of Wisconsin (unpublished study by H. S.), tested on medical students, and found to correlate very well with whether a person learned best in an isolated atmosphere, by reading, or in a large group environment such as a classroom session.

One hundred twenty-five chairmen received the material. Many did not participate in the study because they did not have a large enough group in the town in which the medical school was located to choose the two groups or because they felt that choosing two groups (one being the best practitioners) might disturb town-gown relationships—I understand their concerns. I want to thank very much those chairmen who did participate in the study. I realize it took valuable time to convince their colleagues in town to complete the questionnaire. I also realize that making the chairmen the *sole* judge of who are the best practitioners introduces potential bias, but I know of no objective way to make this judgment. In general, I do feel that the chairman is in a unique position to see which physicians in the hospital or community seem to have the fewest errors in their practice.

Four hundred twenty-nine surveys were returned from 67 different institutions. As you can see (Table I), the two groups were quite equally divided and were primarily in the practice of obstetrics and gynecology. Eighty-five percent of the best group and 74% of the average group had part-time appointments in the medical school, indicating that the chairman had ample opportunity to observe the activities of the participants.

There were some interesting differences in the two groups. Significantly more of the best practitioners were in group practice (Table II). Significantly more of the average practitioners graduated from state schools: 59% compared to 49% in the best group (Table III). Significantly more of the best group were in the upper quarter of their class (Table IV) and were AOA 16% versus 9% ($p < 0.001$). The best group were more likely to have received their residency training in university hospitals (Table V) and had more postresidency training. Twenty-four percent of the best group and 17% of the average group had taken postresidency training ($p < 0.001$). However, most of this postresidency training did not lead to a subspecialty, since only 4% of the

Table VI. Reading of journals

	Best group			*Average group*		
	n	%	*Degree of importance**	*n*	%	*Degree of importance*
AMERICAN JOURNAL of OBSTETRICS and GYNECOLOGY	150	66	2.5	121	60	2.2
Obstetrics and Gynecology	211	92	1.9	174	87	1.9
Journal of Reproductive Medicine	141	62	3.9	106	53	3.4
Contemporary Ob/Gyn	195	85	2.7	152	76	2.7

*Rating scale of 1 to 7, 1 being most important and 7 being least.

Table VII. Meeting attendance

	Best group			*Average group*		
	n	%	*Degree of importance*	*n*	%	*Degree of importance*
Annual meeting	157	68.5	1.8	123	61.5	1.8
District meeting	118	51.5	1.7	92	46.0	1.7
Local or state meeting	168	73.4	5.0	132	68.0	4.4

Table VIII. Degree of importance of factors in selecting postgraduate courses

	Best group	*Average group*
Topic	1.3	1.4
Speakers	2.3	2.7
Location	2.9	2.8
Cost	4.7	4.3
Time and year	4.3	4.1
Length of meeting	4.4	4.2

best group and 1% of the average group stated that they had a subspecialty. Interestingly, over 40% of both groups had done research during their residency. The vast majority of respondents were Board certified, 96% and 88% of the two groups, respectively. Significantly more of the best group had taken the American Board of Obstetrics and Gynecology recertification examination: 38% in the best group versus 29% in the average group ($p < 0.01$).

A look at the postgraduate activities of the two groups revealed essentially no differences in the areas tested regarding reading habits or attendance at meetings. In attempting to assess the importance attached by the respondent to various questions, such as which journals they preferred and other sources of continuing education, we used a rating scale of 1 to 7, 1 being the most important and 7 being the least important.

The questionnaire asked whether they read a number of journals including the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, *Obstetrics and Gynecology*, *JAMA*, *New England Journal of Medicine*, *Journal of Reproductive Medicine*, *Contemporary Ob/Gyn*, *Fertility and Sterility*, *Clinical Endocrinology*, and *Seminars in Perinatology*. The four most widely read are shown in Table VI. *Obstetrics and Gynecology* was the most widely read, 92%, and had the highest importance score, 1.9. *Contemporary Ob/Gyn*, while more widely read than the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, had roughly the same importance score. The average number of articles read per month was 3.6 for the best group and 3.7 for the average group, although among the Board-certified individuals 50% of both groups read seven or more articles per month.

The articles read were chosen primarily for immediate clinical relevance, 59% for both groups, as compared to long-term clinical relevance, 29%, or basic science, 10%. The article to be read was chosen primarily by the title, an importance score of 1.8, as compared to journal, author, or abstract, importance scores of 2.6 to 3.7.

A surprisingly large percentage of individuals attended state, district, or national meetings (Table VII), and about 90% had attended one or more postgraduate courses during the past 3 years. The average number attended was slightly in excess of four. The courses were chosen primarily because of the topic being presented (Table VIII), with speakers and locations of secondary importance. The type of course preferred was one presenting new techniques, with roughly 75% indicating this preference. General review courses were chosen by only 25%.

Finally, we surveyed the textbooks found in their library. The four most commonly found were *Williams Obstetrics*, *Operative Gynecology*, *Clinical Endocrinology and Infertility*, and *Medical Complications of Pregnancy* (Table IX lists the percentages and relative importance of these texts). Their importance ranking was roughly equal to that of the major journals read.

Table IX. Textbooks found in physician's obstetrics/gynecology library

	Best group			*Average group*		
	n	%	*Degree of importance*	*n*	%	*Degree of importance*
Williams' Obstetrics	220	96	2.7	179	94	2.7
Clinical Endocrinology and Infertility	182	79	2.3	129	64	2.5
Operative Gynecology	207	90	2.9	179	89	2.8
Medical Complications of Pregnancy	169	74	2.8	135	67	3.1

Table X. Preferences in postgraduate continuing education for different types of learners

	Best group (%)		*Average group (%)*	
	*Receptive (−5 to −15)**	*Discovery (+4 to +21)*	*Receptive (−6 to −16)*	*Discovery (+3 to +20)*
Journal club	31.4	40.4	17.9	33.3
Hospital staff conference	86.3	83.0	89.7	88.9
University grand rounds	41.2	51.1	43.6	46.7
Reading literature	82.4	95.7	92.3	97.5
Postgraduate courses	88.2	87.2	82.1	93.3
Curbside consultations	45.1	57.4	53.8	62.2
Self-assessment tests	35.3	29.8	35.9	42.2
Conventions	66.7	74.5	64.1	73.3

*Scores according to preferred learning style index (Fig. 1).

The preferred learning style index is a measure of the style of learning preferred. A negative score indicates a receptive-style learner who prefers to encounter the entire content of what is to be learned in the final form. I believe "spoon-fed" is the vernacular. A positive score indicates a discovery-style learner, one who prefers to encounter content in an unstructured way and to gather information. This has been tested by one of the authors (H. S.) on medical students accepted to the University of Wisconsin. There was a very good correlation between the discovery-style learner and the student who chooses independent studies as well as between the receptive-style learner and the one who chooses the regular more traditional curriculum. Fig. 1 shows that there was a Gaussian distribution of the learning style index for the best group. It was similar for the average group. The mean for the best group was 0.57 and for the average group was 1.30. The discovery-type person tended to prefer a journal club, reading the literature, and curbside consultations more than the receptive-type (Table X). Discovery-type persons were more likely to have been in the upper quarter of their class (Table XI) and to read 15 or more articles per month (Table XII).

One must be cautious about the conclusions drawn from a study such as this because the people surveyed are located primarily in a university setting and may have little relationship to what an obstetrician and gynecologist in a rural setting may do for his or her postgraduate education. However, within the constraints of the study it is obvious that all practitioners surveyed make significant use of many postgraduate educational activities. They read more than predicted and they attend postgraduate courses frequently. The fact that there is no difference in the postgraduate activities of the best versus the average group can be explained in a variety of ways. The first way is that the populations may not be different except in the minds of the chairmen. This would seem unlikely, since there were significant differences in type of practice, medical school, class standing, honors in medical school, postresidency training, and recertification. The second explanation assumes that the populations are different and that one group does practice a better brand of medicine than the other. Since both groups have the same postgraduate exposure, a logical conclusion would be that the average group does not benefit to the same degree from their exposure as does the best group. This is not to say that the average group does not benefit from the postgraduate education offered, but they do not seem to benefit to the degree that the best group does unless they start from a lower base of knowledge, which may be the case.

We have not measured changes in practice patterns in this study; however, other studies have attempted to do so with varying degrees of success. Sibley et al.[4] in a study of 16 Ontario family physicians, eight study physicians, and eight control physicians, gave contin-

Table XI. Learning style compared with academic standing

	Best group				*Average group*			
	*Receptive (−5 to −15)**		*Discovery (+4 to +21)*		*Receptive (−6 to −16)*		*Discovery (+3 to +20)*	
Quadrant	*n*	%	*n*	%	*n*	%	*n*	%
Upper	23/50	46.0	29/45	64.4	17/39	43.6	22/43	51.2
Upper middle	23/50	46.0	14/45	31.1	15/39	38.5	16/43	37.2
Lower middle	4/50	8.0	2/45	4.4	6/39	15.4	4/43	9.3
Lower	0	0	0/45	0	1/39	2.6	1/43	2.3

*Scores according to preferred learning style index (Fig. 1).

Table XII. Learning style compared with average number of articles read per month

	Best group				*Average group*			
	*Receptive (−3 to −15)**		*Discovery (+4 to +21)*		*Receptive (−5 to −16)*		*Discovery (+3 to +20)*	
Number of articles read	*n*	%	*n*	%	*n*	%	*n*	%
≤2	1/50	2.0	0/47	0	0/49	0	0/43	0
3-6	8/50	16.0	4/47	8.5	13/49	26.5	5/43	11.6
7-10	22/50	44.0	12/47	25.5	14/49	28.6	12/43	27.9
11-14	9/50	18.0	5/47	10.6	8/49	16.3	7/43	16.3
>15	10/50	20.0	21/47	45.0	14/49	29.0	24/43	55.8

*Scores according to preferred learning style index (Fig. 1).

uing education packages covering commonly encountered practice problems and found that the study physicians learned from the packages but did not necessarily change their quality of care. This is a very interesting study because, while the number of physicians studied was small, it was a well designed, well controlled trial. In the trial each study physician was given a choice of 18 educational packages and then given their first and second choice packages (their high-preference packages). The authors then selected two packages from the unranked list. The study physicians studied all four packages. Chart abstract studies of patient care did not show a significant improvement in the care of patients with preference package conditions but did show a significant improvement in the care of those patients having unranked conditions. Since our own study demonstrated that the foremost reason for choosing a postgraduate course is topic, the data of Sibley et al. would add further doubt that our study participants will alter their practice behavior because of postgraduate courses.

A study demonstrating the effectiveness of continuing medical education was reported by Handler.[5] In this study a problem was identified, too many blood transfusions, and in one hospital over a 15-year period multiple educational conferences were directed toward educating the physicians as to when transfusion is truly indicated. Peer review meetings were held on a frequent basis to discuss inappropriate transfusions. They demonstrated a significant reduction in blood products used in this hospital compared to similar hospitals in the area without such an educational effort.

There are many studies in the literature on the topic of continuing medical education. Haynes et al.[6] collected 248 original articles from the literature and evaluated them for scientific credibility. Only seven studies met all of the authors' criteria for scientific credibility. Three of seven studies assessed patient outcomes, and only one of these demonstrated improvement in outcome. However, in six of the seven studies physician behavior was felt to be significantly influenced. The six studies had at least one thing in common, physician feedback, which served as continual reinforcement, as was demonstrated in Handler's study.

It is readily apparent from our own study as well as many others in the literature that although postgraduate medical education is widely available and broadly utilized, its effectiveness as generally practiced at present is probably very limited. The requirement of many states for X number of postgraduate hours per year for relicensure is inefficient, ineffective, and expensive. Wisconsin, for example, is dropping the requirement. Does this mean that all the national courses should be scrapped and the textbooks and journals abandoned? Definitely not. It is my personal opinion that there could be fewer national postgraduate courses and fewer articles in the literature, with concentration in selecting both the courses and articles of greatest merit. However, restriction of these courses and articles is not the real answer; it is only the beginning.

Revision of the written portion of the American Board of Obstetrics and Gynecology recertification process is not the answer either. The Ontario physicians study demonstrates clearly that you can change the knowledge, as indicated by improved test scores, without necessarily changing practice patterns. However, another part of the recertification process may give some insight into an individual's practice pattern. This is the requirement that a 6-month case list be submitted. The case list is reviewed by two Board members, and if any irregularities are found, the individual is quizzed about the cases. A similar mechanism has been discussed in the time-limited certification process. The advantages of such a system are that it will undoubtedly identify some practitioners who are deficient and that it is a program that can be managed logistically In addition, such a program could identify frequently encountered problems in practice patterns, and these problems could be stressed in our national and local postgraduate efforts. The disadvantage is that it is not a chart review and thus may not identify many practitioners who have poor practice patterns; moreover, it is a periodic rather than ongoing review.

I feel that the best answer will be found at the local level. Peer review committees to monitor specific problems are necessary, and they must be protected from the current legal problems that have rendered so may peer review committees impotent. An excellent example of a current problem that can best be solved in this manner is the rising cesarean section rate. Careful review of all cases of cesarean section because of failure to progress in labor and because of fetal distress has kept the section rate low in at least one study reported recently.[7]

The conclusions are obvious: define the problem, reinforce education continually, and test for changing practice patterns in the individual. This is easy for me to state but difficult to accomplish, yet it must be done if we are to further improve the care of the obstetric and gynecologic patient.

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Ethics in obstetrics and gynecology

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We are now in an era when everything done in medicine is judged in ethical terms, and this is certainly the case for obstetrics and gynecology, which has had more than its share of morally troubling issues. A partial list might include abortion, birth control, dealing with the sexual problems of dependent minors, in vitro fertilization, surrogate parenting, and treating or not treating the multi-handicapped newborn infant and the list goes on.

There are other important, but less publicized and less dramatic moral issues that we share with all of medicine, such as fidelity to science and objectivity, the teaching and learning and doing of research without exploitation of the patient, and not corrupting patient care under the ever-present threat of a malpractice claim.

The judgment of medicine's ethical performance has been made by outside social critics like Freidson[1] or Illich[2] who label doctors moral entrepreneurs. According to them, we throw our weight around authoritatively and manipulate people with our diagnoses and treatment. Judgments have been made by bioethicists like Gorovitz,[3] who thinks: "There is nothing in the training or expertise of the physician which would justify any particular claim to moral authority or even moral insight." The Hippocratic Oath and Codes of Professional Ethics no longer command much respect. There has arisen the notion that each hospital needs a resident ethicist to tell the doctors and nurses what to do, or to tell them how to think morally about what to do, and every hospital needs an ethics committee to lay down moral laws.

Not everyone thinks this makes sense. Caplan,[4] while at The Hastings Institute, wrote, and I agree: "It's naive to think a moral philosopher armed with a moral theory can step into an emergency room or intensive care nursery and immediately dissolve moral conundrums and it's presumptuous to think that health professionals with even modest intelligence would have failed to resolve the quick and easy moral problems."

However, naiveté and presumption have been the order of the day in medical ethics, shared by physicians and bioethicists alike. As we struggle to do the right thing in an open, pluralistic, changing society, let us see what we can and must do. If physicians are to regain any of the lost moral high ground they must at least understand the historical precedents for the moralization of medicine. They must recognize moralizing for what it is, and they must understand enough of moral philosophy and its special jargon to recognize credible moral argument when it is offered, and they must learn to trust their own common sense and judgment.

Good moral reasoning is nothing but good reasoning from a moral perspective. Moralization is the tendency to cast everything we do in moral terms. It can be overdone. It's a form of moral imperialism. For example, in a questionnaire I received, the problem was posed: "At what level of blood pressure would it be *unethical* not to treat the patient?" Imagine what it does to your thinking process when every treatment decision you make is cast oversimplistically in a moral framework.

In another vein, Gorovitz,[5] in a book on medical morality, devoted a chapter to the problems of calling patients by their first names, of keeping patients waiting, of doctors insisting on being called doctor or signing M.D. after their name. These can all be problems of bad taste, inefficiency, overwork, and maybe even arrogance, but as Fletcher[6] says, "Moralism reduces ethics to pettifoggery and trivializes it." We should not trivialize what we do by reducing everything to simplistic moralism.

On a higher plane the most common explanation for the source of increased moral problems in medicine today is the advance of science and technology. There appear to be novel moral problems caused by increased capability to treat and support life, sometimes without cure, and there appear to be new choices: whether to use a respirator, to transplant an organ, to fertilize in vitro. These kinds of choices without the new technology have always been with us in some form of benefit versus burden, benefit versus cost, and distributive justice and patient autonomy. What is new is the success

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of medicine. It creates the impression of being able to do anything, and success and cure have come to be expected. Nothing is chance anymore. If things go wrong, the physician is at fault. There is also an aura of alienation: life without hope (attached to a machine) or life without love (conception without coitus).

Medicine's success is its moral burden, but there is a history of other moral burdens. In the aftermath of World War II, the Nuremberg Trials revealed the immorality of physicians working for the German state. Their cruel research resulted in a code of ethics after the allied victory.[7] Other studies revealed that doctors working for themselves in the prewar era also did research without consent, even with grave hazard to their patients. It seemed to predispose them for the barbarism of the concentration camps, for example, transplanting breast cancer from one side to another to see if it took. It did.

Even in the United States, years after Nuremberg, research at the Jewish Memorial Hospital and Willow Brook raised grave questions about how well physicians, doing clinical research, could monitor themselves and protect their subjects. The medical ethics that was supposed to protect was found wanting.[8]

During the past ten years, the National Commission on Ethics, which I chaired, and its successor, the Presidential Commission, chaired by Morris Abrams, were attempts to develop some sort of national consensus for dealing with moral problems in research and medical practice. With the commissions have come some public discourse and regulation.[9]

In the 1960s, there were cultural upheavals. Society decided to push for civil rights and women's rights. Authority and secrecy were attacked in government, in big business, and, yes, even in medicine. There was public accommodation to a freer sexuality, which was more explicit and less "responsible," and there was a tolerance for alternative life-styles.

Remember how this new morality influenced the image of national political parties and note today the backlash with an emphasis on morality and the family.

Moralism has become the order of the day, and it is different than the moralism of the 1960s. Do not think for a moment that this does not influence how medicine is practiced. Medical practice has changed with the times. We are more defensive about technology and fetal monitoring and the use of forceps and cesarean sections. We have a love-hate relationship with this technology. We are criticized if we use it and we are sued if we do not use it and something goes wrong. Patients' rights have been written into statute and our so-called moral choice is fading and with it goes the sense of moral responsibility that we need.

There is still more social history as recorded by the late French sociologist Foucault.[10] This is the moralization of sex from the seventeenth century through the Victorian era. We went from frankness about sexual practices to silence and secrecy. Sex became utilitarian and licit only for procreation. It was wrong to talk about sex in polite society, but it was always on people's minds.

The science of reproductive biology in animals could progress almost normally, but the medicine of sex foundered. There have been two centuries of mistaken notions about the neurotic sexuality of women, of the evils of masturbation, of the norm of being a Malthusian procreative couple, and of settling sexual perversion on the psychiatrist's couch. This is what was fought against in the 1960s. Much of what was thought and practiced in obstetrics and gynecology was influenced by these social attitudes toward sex which doctors helped to shape. Morality was misplaced on the stereotyped form of sex (what people did) and not enough attention was focused on what was important (how people care for one another). Please note that giving birth control information to patients was illegal in the state of Massachusetts in which I went to medical school. In a neighboring state to the south, a professor was hailed into court for providing birth control information.

These are the traditions and events that shaped people's behavior and attitudes in and out of medicine: two centuries of sexual prudery and hypocrisy, the holocaust and Nuremberg, and the cultural upheavals of the 1960s that challenged the old sexual moralism and made politics and business and medicine moral issues. The upsurge of bioethics in the 1970s provided jobs for philosophers with few other opportunities, the national commissions fostered public discourse, and finally medical science successfully prolonged, maintained, and created life, all against the tapestry of our inherited religious and cultural traditions in a melting pot society.

Moral problems arising out of abortion or treating the handicapped newborn infant or in vitro fertilization cannot be truly understood or even intelligently approached without a sense of this history. Any ethical analysis that neglects prior human experience is bound to be superficial or abstract. Without knowing the historical story one may not even know what the problem is or whether there is a problem.

One must seek the facts of the matter. Every moral problem has a history of relevant human experience, a history of ends sought, of means used, of consequences, sometimes differing between cultures and over time.

Moral concepts can, however, change as social life changes. We have had more than a decade of experience with Baby Doe cases in modern nurseries and a vast history of infanticide since the time of the Greek city states. We have had commentary on in vitro fertilization for years and *Brave New World* was written in 1931. The Supreme Court looked at 2500 years of abortion in its *Doe vs. Wade* decision. For someone to make an unqualified self-certifying moral judgment about either side of the abortion issue is like someone saying the world is flat. You can assert it, but it just may not square with the "facts." What do I mean by facts?

It may seem strange or wrong to think about facts in morality and in relation to what is right or good. Moral philosophers preach the separation of facts and value and the separation of what is and what one ought to do—and they are right in the abstract but not in the real world. They have neglected the descriptive ethics of what people really do.

Dewey[11] clearly pointed out the distinction of the value of something and how one judges value by valuing. It is in the values and the valuing that we develop historical facts about past human experience. This provides a basis for prediction about the future and may influence present choices. We have to understand why and how we value things and history provides an important link that abstract moral theory does not.

To seek facts is commendable, but to see the world as it really is, is the key to morality. As MacIntyre[12] put it: "Objectivity was a moral concept before it became a scientific one." A good deal of intractable dispute on both sides of moral issues revolves around our inability to see things clearly because of biases and emotion.

I see no easy path to see the world as it really is except to exhort people to pursue facts objectively, to argue them openly, to define words, and to try to recognize where logic (consistency of argument) and the reasoning process part company. Few moral philosophers talk about this concept of getting facts and seeing the world as it is and that is their chief failing, substituting abstract theory for moral facts.

One who does champion this concept of trying to see the world as it is, is the novelist and philosopher Murdoch.[13] It is not simply "give me the facts and I can then make a moral judgment," it is "I need moral guidance in seeking and seeing the facts."

A special requirement of ethics is to treat like cases similarly so that moral analysis means looking for morally relevant differences or similarities. I was struck by the apparent uniqueness and lack of precedent on what to do with an unused frozen embryo, until I started to look for moral relevance to the status of life at that stage in and out of the womb and the history of how people value life at less than two weeks' gestation. There is no single right answer but what starts as a seemingly arbitrary and unique problem becomes merged into the pattern of past experience and can be approached with understanding.

Most moral philosophers like to work from single moral theories, and they do not like people to be catholic in using more than one approach.

There are the teleologists or utilitarians, who look at ends or the "good" of the outcome. There are the formalists, or ontologists, or legalists, or rightists that work from rules or means or the right of any action, not the outcome.

A good many philosophers bend over backward to remain true to one approach or another which is really pursuing rigorous argument for its own sake and not its utility in the real world. Real people in the real world look at both ends and means, at consequences and rules, at both what is good and what is right. When the good and the right conflict, we have a choice to make. Philosophers like to close out choice at that level and they are wrong. Dewey championed the means-ends continuum and the value of looking at experience, which is why I so admire his work.

Although Kant[14] is a towering giant in philosophy and advocated treating people as ends in themselves and not as means, he was a rigid rule follower. He wrote an essay, entitled "On a supposed right to tell lies from altruistic motives." To Kant, there was no right to tell lies even to hide a victim from a would-be murderer. That is why I have to take Kant in the context of his world, his hang-ups and his morality that cannot possibly be the same as mine.

Incredibly, unimaginative theoretical philosophers still choose up sides on the means-ends dispute.

The arguments that bioethicists sometimes make revolve not around the single case and its means and ends but on the consequences that will ensue. Hence you will hear arguments like, if we allow euthanasia, people cannot be trusted to pick the right cases, or, if we allow in vitro fertilization, it will lead to cloning, if we allow birth control, it will lead to abortion, if we allow abortion, it will lead to infanticide, and so on. "It might be alright in this one extreme case, but we can't do it because of what could happen if it became a precedent."

This is the slippery slope, camel's nose in the tent, entering wedge arguments that are insidious. The argument is itself an opening wedge. For Fletcher: "Abusus non tollit usum" (Abuse does not bar use).[6]

I have not tried to settle any unanswered moral questions in this presentation. When we go public, we do no more than simply try to decide how we will live together rather than set moral issues once and for all. The public, social, political process unfortunately encourages superficial moral analysis, under the glare of television cameras and the sensation-seeking media.

To make moral judgments, one may not need the so-called moral expert, but one needs to pay attention to at least the following:

1. Seek facts. Do not try to make a moral "diagnosis" and apply moral "treatment" without taking a case history.
2. Remember that moral concepts may change.
3. Purge fact finding of bias and emotion. Try to see the world as it is. True science is the model, not the findings of science but the method of science.
4. Look for morally relevant cases in trying to analyze a problem. There is nothing new under the sun.
5. Consider both the good and the right. Do not be afraid to look at both means and ends. If you want to be a utilitarian or a deontologist (rule follower), fine, but you do not have to be one or the other. If you follow religious traditions remember religion itself has a history and has facts. Seeking religious facts is like trying to see the world as it is. Even religious practices and beliefs change as morality and society change.
6. Wedge arguments have to be tested against whether abuse is inevitable. Remember there is essentially no human action that cannot be abused, except perhaps Kant's "Goodwill."

Finally, what I have described in plain language is more or less from the tradition of American pragmatism, a philosophy that has tried better than any other to accommodate human values to the advance of technology.

Note the words of the philosopher James.[15] A pragmatist turns his back resolutely and once for all upon a lot of inveterate habits dear to professional philosophers. He turns away from abstraction and insufficiency, from verbal solutions, from bad a priori reasons, from fixed principles, closed systems, and pretended absolutes and origins. He turns toward concreteness and adequacy, toward facts, toward action, and toward power.

James occasionally had an infelicitous way with words. I like to think that the power he is talking about is not the exercise of power, but the consequence of doing good in the world. That should be something we can all agree upon.

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Relationship of fetal oxygen consumption and acid-base balance to fetal hematocrit

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We evaluated the effects of alterations in fetal hematocrit on fetal oxygenation in 10 chronically catheterized fetal lambs. Hematocrit was varied from 10% to 55% by slow isovolemic exchange transfusions with plasma or packed red blood cells obtained freshly from donor fetuses. At each hematocrit studied, we measured umbilical blood flow ($\dot{Q}_{umb}$) and the oxygen concentrations in umbilical venous blood (Cuv_{O_2}) and arterial blood (Ca_{O_2}) and calculated fetal oxygen delivery ($\dot{Q}_{umb} \cdot Cuv_{O_2}$), oxygen extraction [$(Cuv_{O_2} - Ca_{O_2})/Cuv_{O_2}$], and oxygen consumption [$\dot{Q}_{umb}\,(Cuv_{O_2} - Ca_{O_2})$]. Fetal oxygen delivery was maximal at a fetal hematocrit of 33% (mean oxygen delivery = 23 ml of oxygen per minute per kilogram of fetus) and decreased as hematocrit was raised or lowered from that value. Despite these reductions in oxygen delivery, fetal oxygen consumption was relatively stable (at about 7 ml of oxygen per minute per kilogram) at hematocrits ranging from about 16% to 48% because of compensatory increases in fetal oxygen extraction. Regardless of whether oxygen delivery decreased because of anemia or polycythemia, fetal oxygen consumption was maintained as long as oxygen delivery was greater than about 14 ml of oxygen per minute per kilogram of fetus. When oxygen delivery was <14 ml of oxygen per minute per kilogram, fetal oxygen consumption fell while arterial blood base deficit increased, indicating that oxygen supply was inadequate for fetal oxygen demands. These results indicate that fetal aerobic metabolism can be sustained over a wide range of fetal hematocrits. Furthermore, our data support the concept that the level of fetal oxygen delivery is an important determinant of the adequacy of fetal oxygenation. (AM J OBSTET GYNECOL 1985;151:844-51.)

During pregnancy, several maternal and fetal complications are known to be associated with alterations in fetal hematocrit.[1-3] These complications include conditions as varied as diabetes mellitus, fetal growth retardation, rhesus isoimmunization, and the twin-to-twin transfusion syndrome. The resultant changes in fetal hematocrit and thus hemoglobin concentration produce alterations in both the oxygen concentration and the viscosity of fetal blood.[3, 4] In adult animals, each of these two variables has been shown to affect perfusion and oxygen supply to individual organs as well as to the organism as a whole.[5, 6] Although these effects must theoretically occur in the fetus as well, there are very few experimental data describing the effects of hematocrit variations on fetal oxygenation.

Tenenbaum et al.[4] found that oxygen delivery to the fetal lamb (umbilical blood flow × umbilical venous blood oxygen concentration) decreased when fetal hematocrit was increased 40% above normal. Even though fetal oxygen delivery decreased, the quantity of oxygen consumed by the fetus was maintained by compensatory increases in fetal oxygen extraction. Their studies did not identify the limits of the fetal compensatory response to polycythemia because only one level of polycythemia was studied.[4] It is likely that if hematocrit had been increased further the limits of compensation would have been exceeded and the rate of fetal aerobic metabolism would have decreased. Furthermore, no studies have been done to define the extent of the fetal adaptive response to anemia. In view of the many clinical conditions associated with fetal anemia and polycythemia,[1-3] it would be important to determine the range of hematocrits over which fetal aerobic metabolism can be sustained. The purpose of our studies was to identify the fetal compensatory responses to varying degrees of anemia and polycythemia and to define the limits of those responses.

Methods

Preparation of animals. We prepared 10 fetal lambs ranging in gestational age from 100 to 130 days (term = 147 days). The surgical techniques used have been described in detail[7, 8] and will be summarized only. After an epidural or spinal anesthetic (tetracaine hydrochloride, 1%) was administered to the mother, we opened the maternal abdomen and uterus and placed

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catheters into a fetal hind limb artery and a vein. These catheters were advanced into the descending aorta and inferior vena cava. Anesthesia for catheter placements in the fetus was provided by lidocaine hydrochloride, 0.5%, administered locally. We then incised the uterus over the area of the fetal abdomen and inserted a catheter into the common umbilical vein. A catheter was also placed in the amnionic sac. In eight of the 10 fetuses, twins were present; in these cases, we placed catheters into a hind limb artery and a vein in the second twin. We used this second twin as a plasma or red blood cell donor. The maternal abdomen was closed and all catheters were brought out subcutaneously to the ewe's flank where they were stored in a pouch. Two to 5 days after operation, we studied the fetuses of the unanesthetized sheep as the sheep stood unrestrained in a transport cage.

Experimental protocol. In each fetus, we measured umbilical blood flow, umbilical venous and arterial blood oxygen concentrations and acid-base balance and calculated fetal oxygen delivery, oxygen extraction, and oxygen consumption at various randomly selected fetal hematocrits ranging from 10% to 55%. To change the fetal hematocrit, we performed isovolemic exchange transfusions by infusing plasma or packed fetal red blood cells through one fetal catheter while withdrawing fetal blood at the same rate from a second catheter.[8] Hematocrits were changed slowly over 1 to 4 hours, during which time fetal arterial blood pressure and heart rate were continuously monitored and recorded. Both variables were stable throughout the study. Plasma for exchange transfusion was obtained freshly either from the donor twin or from an adult sheep, whereas fetal red blood cells were obtained from a donor twin only.

When hematocrit was shown to be stable, we withdrew 0.8 ml blood samples simultaneously from the descending aorta and umbilical vein for the analyses of blood gases, pH, base deficit, hematocrit, and oxygen concentration. Blood gases, pH, and base deficit were measured or calculated at 38° C with a blood gas meter (Instrument Laboratories or Corning), hematocrit was determined in duplicate with the microcapillary technique, and blood oxygen concentration was quantified with a Lex-O_2-Con oxygen analyzer.[7] Immediately after obtaining the blood samples, we injected 1 to 2 × 10^5 radionuclide-labeled microspheres (15 μm diameter, labeled with either iodine 125, cerium 141, chromium 51, strontium 85, niobium 95, or scandium 46, New England Nuclear or 3M Co.) into the fetal inferior vena cava while withdrawing a reference blood sample from the descending aorta at 3.9 or 7.9 ml/min.[7,9] We used the higher withdrawal rate in the anemic fetuses to ensure that blood flow measurements were accurate.[10] Microsphere injections and blood withdrawals did not affect fetal arterial blood pressure or heart rate.[9] The volume of blood withdrawn was replaced with a comparable volume of fetal blood so that a constant intravascular volume could be maintained. After obtaining the oxygen and blood flow measurements at this hematocrit, we changed fetal hematocrit to a new level and repeated the flow and oxygen measurements. Each fetus was studied at an average of three (range, two to five) randomly chosen hematocrits.

In preliminary experiments on two fetuses, we increased hematocrit first and decreased it second; in two additional fetuses we reversed this order. We found no differences in the responses of fetal oxygenation relating to the order in which fetal hematocrits were changed. Therefore, in the remaining fetuses, we either raised or lowered hematocrit; none of these fetuses were studied at both high and low hematocrits. Using this approach, we could considerably reduce the time involved in varying the hematocrit with exchange transfusions.

Preparation of tissues and calculation and analyses of data. When the study was completed, sheep and fetuses were killed with an overdose of sodium pentobarbital. The placenta was removed and the amounts of each radionuclide present in it and in the reference blood samples were measured with standard methods.[9] We computed umbilical (fetal-placental) blood flow ($\dot{Q}_{umb}$) as follows:

$$\dot{Q}_{umb} = (cpm_{plac}/cpm_{ref})\dot{Q}_{ref}$$

where cpm_{plac} = radioactive counts per minute in the placenta, cpm_{ref} = radioactive counts per minute in the reference arterial blood sample, and $\dot{Q}_{ref}$ = reference arterial blood flow determined by calibrating the withdrawal pump. $\dot{Q}_{umb}$ was expressed as milliliters per minute per kilogram of fetal body weight.

We calculated three variables of fetal oxygenation, with the use of modifications of the Fick principle[11,12]:

$$\text{Fetal oxygen delivery (fetal } Do_2) = \dot{Q}_{umb}\,(\mathrm{Cuv}_{O_2})$$

$$\text{Fetal oxygen consumption (fetal } \dot{V}o_2) = \dot{Q}_{umb}(\mathrm{Cuv}_{O_2} - \mathrm{Ca}_{O_2})$$

$$\text{Fetal oxygen extraction} = \frac{\text{fetal } \dot{V}o_2}{\text{fetal } Do_2} = \frac{\dot{Q}_{umb}\,(\mathrm{Cuv}_{O_2} - \mathrm{Ca}_{O_2})}{\dot{Q}_{umb}\,(\mathrm{Cuv}_{O_2})} = \frac{\mathrm{Cuv}_{O_2} - \mathrm{Ca}_{O_2}}{\mathrm{Cuv}_{O_2}}$$

where Cuv_{O_2} = umbilical venous blood oxygen concentration and Ca_{O_2} = arterial (fetal descending aortic) blood oxygen concentration, in milliliters of oxygen per deciliter of blood. Fetal Do_2 and $\dot{V}o_2$ were expressed as milliliters of oxygen per minute per kilogram of fetus.

We plotted the following dependent variables against fetal hematocrit: umbilical venous and arterial blood oxygen concentrations and oxygen tensions, umbilical

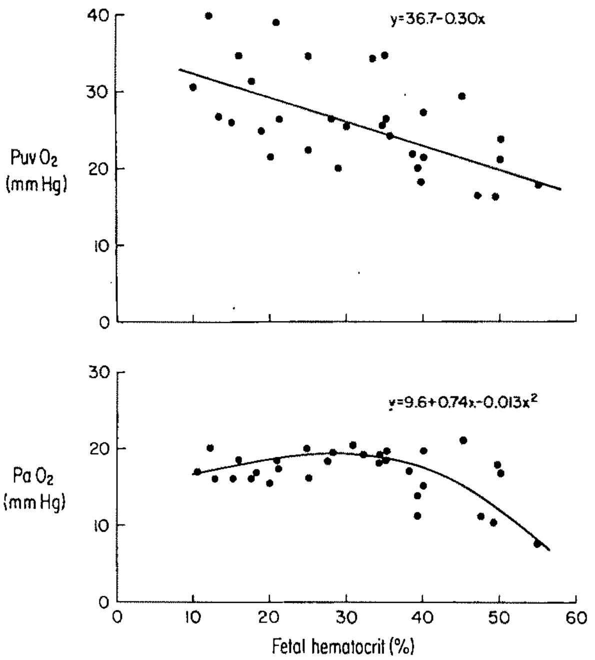

Fig. 1. Umbilical venous blood P_{O_2} (*$PuvO_2$, top*) and fetal arterial (descending aortic) blood P_{O_2} (*PaO_2, bottom*) as functions of fetal hematocrit. In this and subsequent figures, data were obtained from 10 fetal lambs and were analyzed with analysis of covariance. The regression lines that were drawn take into account responses observed in individual fetuses.

blood flow, and fetal oxygen delivery, oxygen extraction, and oxygen consumption. Data analyses were based on 31 measurements made in the 10 fetuses studied. We analyzed our data with a least-squares method based on analysis of covariance, with which we could generate regression lines that took into account responses seen in individual animals.[13] Using this method, we could define mathematically the relationships that existed between the various dependent variables and fetal hematocrit. In most cases, equations of the general form, $y = a + bx + cx^2$ or $y = a + b \ln x$, had the greatest correlation coefficients with the least residuals and thus best described these relations. When quadratic or logarithmic functions did not provide the best fit, linear correlation analyses were done on the raw data.[14]

Results

On the day of study, fetal lambs were 118 ± 7 days of gestation (mean ± SD), and fetal body weight was 2.1 ± 0.6 kg. Hematocrit before the start of the study was 32% ± 3%. Fetal heart rate and arterial blood pressure (mean and systolic or diastolic) varied <15% from baseline values over the entire range of hematocrits studied (10% to 55%); neither heart rate nor blood pressure was related to fetal hematocrit. Umbilical venous blood P_{O_2} decreased linearly ($p < 0.005$) as hematocrit was increased from 10% to 55% (Fig. 1). In contrast, fetal arterial blood P_{O_2} did not change significantly until hematocrit was raised above about 40%, at which point arterial P_{O_2} fell ($p < 0.005$). Umbilical venous blood oxygen concentration increased linearly ($p < 0.005$) with hematocrit (Fig. 2), whereas fetal arterial blood oxygen concentration was greatest at a fetal hematocrit of approximately 34%. At higher and lower hematocrits, arterial blood oxygen concentration decreased.

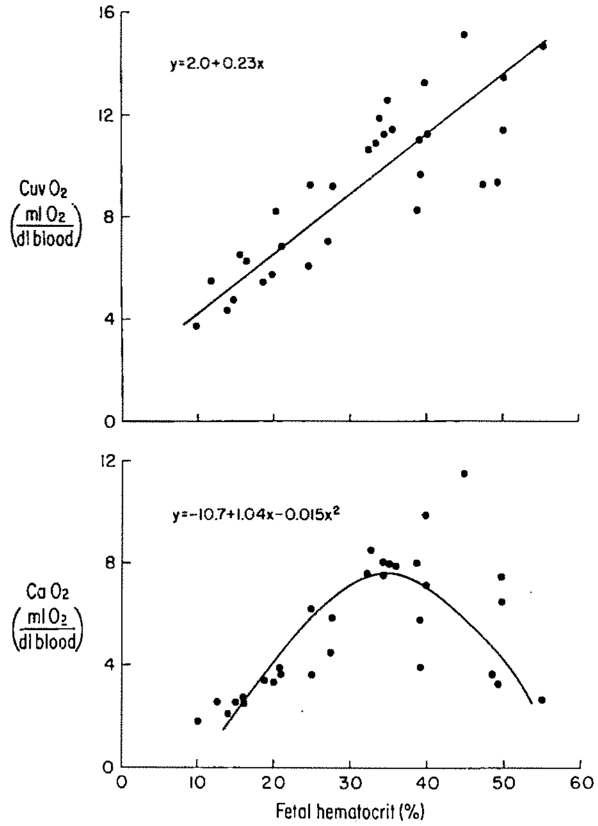

Fig. 2. Umbilical venous blood oxygen concentration (*$CuvO_2$, top*) and fetal arterial (descending aortic) blood oxygen concentration (*CaO_2, bottom*) as functions of fetal hematocrit. Cuv_{O_2} and Ca_{O_2} are expressed as milliliters of oxygen per deciliter of blood.

Fig. 3 shows that umbilical blood flow was relatively unaffected by variations in hematocrit from 10% to approximately 40% but fell at greater hematocrits ($p < 0.01$). Because of the relationships of umbilical venous blood oxygen concentration and umbilical blood flow to fetal hematocrit, fetal oxygen delivery was maximal at a hematocrit of 33% (optimal hematocrit[5, 15]). We calculated the optimal hematocrit by determining the first-order differential equation (dy/dx) relating fetal oxygen delivery (y) to hematocrit (x) and by solving for x when dy/dx = 0. When hematocrit was increased above or decreased below the optimal hematocrit, oxygen delivery to the fetus declined (Fig. 4,

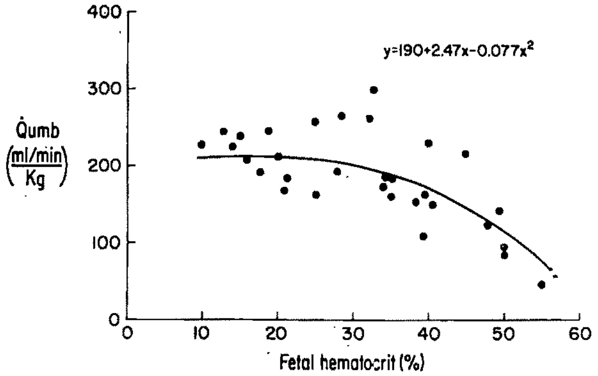

Fig. 3. Relationship of umbilical blood flow ($\dot{Q}_{umb}$, in milliliters per minute per kilogram of fetal body weight) to fetal hematocrit.

top). As compensation for reductions in oxygen delivery that occurred as hematocrits were raised or lowered from the optimum, fetal oxygen extraction (Fig. 4, *middle*) increased sufficiently to enable fetal oxygen consumption to be relatively constant over the range of hematocrits from about 16% to 48% (Fig. 4, *bottom*).

We were also interested in identifying which of the two variables, oxygen delivery or hematocrit, was more important in predicting the adequacy of fetal oxygenation. Using multiple regression analysis, we found that fetal oxygen delivery, rather than fetal hematocrit, was the better predictor of the level of fetal oxygen consumption (the sum of squares of oxygen consumption versus oxygen delivery was greater than the sum of squares of oxygen consumption versus hematocrit[15]). At a fetal oxygen delivery of greater than approximately 14 ml of oxygen per minute per kilogram of fetal body weight, fetal oxygen consumption was unaffected by changes in oxygen delivery (Fig. 5, *top*). When oxygen delivery was reduced below about 14 ml of oxygen per minute per kilogram, regardless whether this response was secondary to anemia or to polycythemia, oxygen consumption by the fetus decreased. Oxygen consumption fell in association with increases in the base deficit in arterial blood (Fig. 5, *bottom*), indicating that fetal oxygen supply was inadequate for fetal oxygen demands.

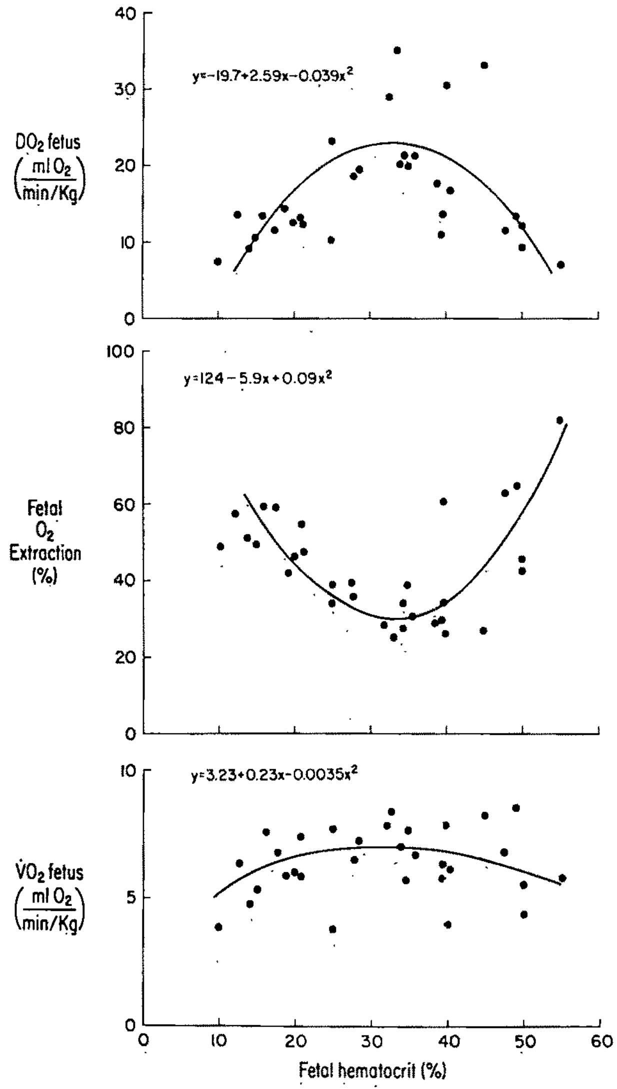

Fig. 4. Fetal oxygen delivery (*DO_2 fetus, top*), fetal oxygen extraction (*middle*), and fetal oxygen consumption (*$\dot{V}O_2$ fetus, bottom*) as functions of fetal hematocrit. Fetal Do_2 and $\dot{V}o_2$ are expressed as milliliters of oxygen per minute per kilogram of fetal body weight.

Comment

The data presented in this report establish that fetal oxygen consumption can be maintained over a wide range of fetal hematocrits. In our studies, aerobic metabolism by the fetal lamb was sustained by compensatory increases in oxygen extraction as oxygen delivery decreased. Our data indicate that regardless whether fetal oxygen delivery dropped as a consequence of anemia or as a result of polycythemia, fetal oxidative metabolism was unaffected as long as oxygen delivery remained above a certain minimum, approximately 14 ml of oxygen per minute per kilogram of fetus. This value is about the same as has been reported in studies in which other methods were used to reduce fetal oxygen delivery.[11, 12] In those studies, when either uterine blood flow[11] or umbilical blood flow[12] was reduced, fetal oxygen consumption did not change significantly until oxygen delivery fell to about 14 ml of oxygen per minute per kilogram of fetus (0.6 mmol/L of oxygen per minute per kilogram[11]). When oxygen delivery fell below that level, fetal oxygen consumption decreased, while the base deficit in fetal arterial blood increased, indicating the development of anaerobic metabolism due to tissue hypoxia. When our results are considered with those just cited, they support the concept that the level of fetal oxygen delivery, rather than the specific

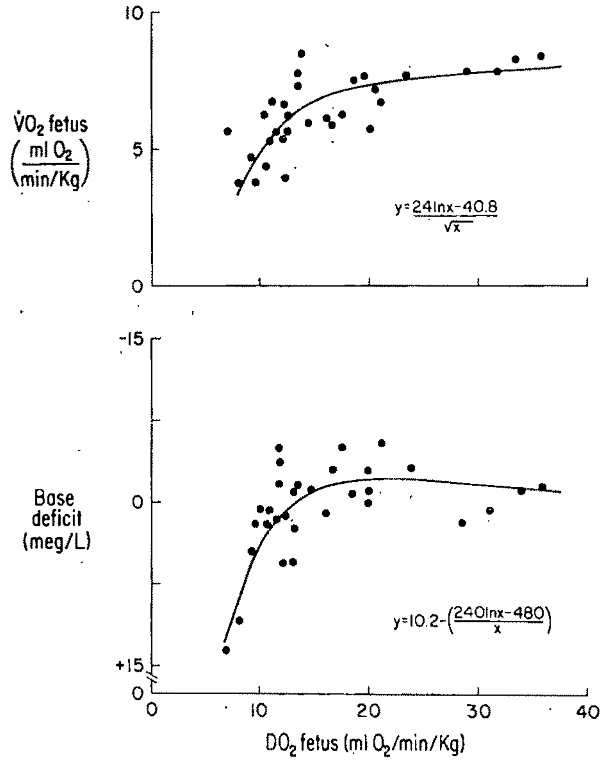

Fig. 5. Relationships of fetal oxygen consumption *($\dot{V}O_2$ fetus, top)* and arterial blood base deficit *(bottom)* to fetal oxygen delivery *(DO_2 fetus). Fetal* $\dot{V}O_2$ and DO_2 are expressed as milliliters of oxygen per minute per kilogram of fetal body weight; base deficit is in milliequivalents per liter of blood.

factor used to affect oxygen delivery adversely, is more important in predicting the adequacy of fetal oxygenation.

Oxygen delivery to the fetus is a function of two variables, umbilical blood flow and the oxygen concentration of umbilical venous blood. At the hematocrit associated with maximal fetal oxygen delivery (optimal hematocrit[5, 15]), we found that fetal oxygen delivery averaged 23 ml of oxygen per minute per kilogram of fetus, a value considerably in excess of the minimum of about 14 ml of oxygen per minute per kilogram necessary for aerobic metabolism. It is of particular interest that the optimal hematocrit we calculated (33%) was about the same as the hematocrit normally found in the fetal lamb (32%).[4, 7] In adult animals, previous investigators[5, 6] have observed similar close relationships of optimal hematocrit to the hematocrit normally present in those animals. These observations suggest that, in the fetus as well as in the adult, maintenance of a hematocrit at or near the optimum for oxygen delivery is an important means of ensuring that oxygen supplies to all tissues are adequate for oxidative metabolism.

In our studies, when hematocrit was decreased from the optimum, fetal oxygen delivery fell, because the oxygen concentration in umbilical venous blood decreased as fetal hemoglobin concentration and blood oxygen–carrying capacity declined, while umbilical blood flow did not change. When we increased fetal hematocrit above the optimal hematocrit, fetal oxygen delivery also decreased, but this decrease was due to a reduction in umbilical blood flow that was proportionally greater than the increase in umbilical venous oxygen concentration due to the rise in fetal hemoglobin concentration. The reduced umbilical flow most likely resulted from increases in blood viscosity that have been shown to occur during fetal polycythemia. Tenenbaum et al.[4] showed that umbilical blood flow fell when fetal hematocrit and viscosity were increased secondary to isovolemic exchange transfusion with adult red blood cells.

Although we noted that umbilical venous blood oxygen concentration increased with increasing fetal hematocrit, the rise in blood oxygen concentration was less than expected, because umbilical venous blood P_{O_2} (and thus oxygen saturation[11]) fell linearly as hematocrit was raised. The reduction in umbilical venous P_{O_2} with increasing hematocrit may have resulted from a change in the relationship of maternal to fetal blood flows within the placenta. Such a change would represent the placental equivalent of an alteration in the matching of ventilation to perfusion in the adult lung. In accord with this idea, variations of hematocrit in the adult dog have been shown to affect arterial blood P_{O_2} inversely, an effect that is due to alterations in the relationship of ventilation to perfusion.[16]

In contrast to our observation that umbilical venous blood oxygen concentration continued to increase with increasing hematocrit, fetal arterial blood oxygen concentration was maximal at a hematocrit of about 34% and decreased at hematocrits above and below that value. The decreases in fetal arterial blood oxygen concentration reflected the increases in oxygen extraction that occurred as compensations for the reduced oxygen delivery of the anemic and polycythemic states. This relationship can be seen if we examine the equations describing fetal oxygen extraction:

$$\text{Fetal oxygen extraction} = \frac{\text{fetal } \dot{V}_{O_2}}{\text{fetal } D_{O_2}}$$

where fetal $\dot{V}_{O_2}$ = fetal oxygen consumption and fetal D_{O_2} = fetal oxygen delivery. Since

$$\frac{\text{Fetal } \dot{V}_{O_2}}{\text{Fetal } D_{O_2}} = \frac{C_{UV_{O_2}} - C_{A_{O_2}}}{C_{UV_{O_2}}}$$

by rearranging these latter equations, we find that

$$C_{A_{O_2}} = C_{UV_{O_2}}\left(1 - \frac{\text{fetal } \dot{V}_{O_2}}{\text{fetal } D_{O_2}}\right)$$

where CUV_{O_2} = umbilical venous blood oxygen concentration and CA_{O_2} = fetal arterial blood oxygen concentration.

During anemia, CA_{O_2} decreases because fetal oxygen extraction increases ([1 − (fetal $\dot{V}O_2$/fetal DO_2)] decreases) while CUV_{O_2} decreases as fetal hemoglobin concentration falls. During polycythemia, CA_{O_2} also falls, because the increase in fetal oxygen extraction (the decrease in [1 − (fetal $\dot{V}O_2$/fetal DO_2)]) is proportionally greater than the associated increase in CUV_{O_2} due to the rise in fetal hemoglobin concentration. Although fetal arterial blood oxygen concentration decreased, there was no change in the arterial blood base deficit over the range of fetal hematocrits from abut 16% to 48%, indicating the absence of tissue hypoxia, anaerobic metabolism, and acidemia. At hematocrits <16% or >48%, which corresponded roughly to a fetal oxygen delivery less than about 14 ml of oxygen per minute per kilogram of fetus, arterial blood base deficit increased in association with reductions in fetal oxygen consumption, confirming that oxygen delivery to some fetal tissues was inadequate for tissue oxygen demands.[11]

In view of our findings, we might speculate on the effects of anemia and polycythemia on the oxygen requirements of the human fetus. Numerous clinical conditions are associated with the development of fetal anemia or polycythemia.[1-3] If the responses of the human fetus to changes in hematocrit are qualitatively similar to those of the lamb fetus, then anemia or polycythemia would cause a reduction in fetal oxygen delivery. During severe anemia or polycythemia, this reduction in oxygen delivery could result in tissue hypoxia and a decrease in fetal oxidative metabolism. Such responses could help to explain why perinatal morbidity and mortality rates are greater in anemic and polycythemic infants[1-3] than in infants with normal hematocrits.

In summary, the results of our experiments show that aerobic metabolism in the fetal lamb can be maintained over a wide range of fetal hematocrits. The principal compensatory response observed was an increase in oxygen extraction when fetal oxygen delivery decreased as a result of alterations in hematocrit. Our results also indicate that, regardless whether fetal oxygen delivery fell as a result of anemia or of polycythemia, fetal oxygenation was normal as long as oxygen delivery remained above a certain level. These data therefore support the concept that the level of fetal oxygen delivery, rather than the specific condition that may be adversely affecting oxygen supply, is the important determinant of the adequacy of oxygenation in the fetal lamb.

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Discussion

DR. JOHN PATRICK, London, Ontario, Canada. Was your experimental design the same for fetuses with normal hematocrits as it was for fetuses with low or high hematocrits? This concerns me because the experiment itself could alter many fetal controls, particularly cardiovascular and hormonal controls. Such alterations could considerably alter overall oxygen consumption.

Second, I think your data might be relevant to fetal anemia during human pregnancy but I am worried that the injection of blood with a high hematocrit to a normoxic fetus does not really reflect the kind of situation we see in growth-retarded fetal lambs or humans. Could you comment on how your observations might relate to a probable change in the distribution of the fetal circulation under conditions of fetal growth retardation. We have certainly all seen fetal lambs that were small and growth retarded, that had high hematocrits with normal oxygen content and low PO_2, and that exhibited normal cardiovascular and brain patterns.

Dr. Robert C. Cefalo, Chapel Hill, North Carolina. Recently we performed an isovolemic exchange transfusion of the fetus with the erythrocyte substitute Fluosol-DA and brought down the hematocrit to levels similar to those you saw.

The respiratory/cardiovascular system parameters measured in the fetus all remained within the normal range even with the hematocrit as low as 10% or 12%.

Interestingly, whether the maternal ewe was hyperoxygenated or in ambient air, in the fetus the PO_2 did not increase above that which we would see in a normovolemic fetus with a hematocrit of 34% even though the hematocrit was in the range of 10 vol/100 ml.

My question would be what was the state of the mother? Did she receive ambient air or was she oxygenated?

Dr. Edgar L. Makowski, Denver, Colorado. Dr. Edelstone, there is one question that I have, and I noted the data were conspicuously absent from your organ flows.

Have you had the opportunity to quantitate hepatic flow under these circumstances? I realize how difficult that is because of the complexities of the hepatic vascular bed.

Dr. Seymour L. Romney, New York, New York. Are you saying basically that the fetus in utero has a totally oxidated metabolic commitment comparable to that of the adult and that there are no differences between adult oxygenated metabolism and that of the fetus, which I thought was anaerobic metabolism? If this is so, what is the range of change in pH?

Dr. Thomas H. Kirschbaum, Bethesda, Maryland. Dr. Edelstone, in the illustration in which you plotted fetal VO_2 as a function of hematocrit, it was your contention that oxygen consumption declined at hematocrit levels >50%. It seemed to me that conclusion was based on a single data point which may have stemmed from the decision to use a parabola to fit the aggregate of data more than anything else.

Would you comment on the certainty that underlies that?

Dr. Jack N. Blechner, Farmington, Connecticut. Dr. Edelstone, I think you have clearly elucidated the aspect of the fetal ability to compensate for physiologic deficiency.

My question is what do you think is the mechanism of the compensatory increase in fetal oxygen extraction for the fetus as a whole above critical oxygen delivery levels? Do you have any thoughts on that?

Dr. Edelstone (Closing). The fetuses we used in our experiments recovered well from operation as indicated by normal respiratory gases, pH, and hematocrits in both arterial and umbilical venous blood. There were, however, two growth-retarded fetuses (which are not included among the results I presented today) that did not recover well from operation. After operation they had high hematocrits and relatively low umbilical arterial and venous blood PO_2 values. The blood pH, PCO_2, and base deficit in these animals, however, were normal. Since we were interested in the relationship of hematocrit to fetal oxygenation in normally grown as well as in undergrown fetuses, we studied these two animals, as follows. We measured umbilical blood flow and fetal oxygen delivery, oxygen extraction, and oxygen consumption at the initial high hematocrits and at several other hematocrits ranging down to 20%. Fetal hematocrit was reduced by isovolemic exchange transfusions with plasma obtained from a donor fetus (see Methods). As hematocrit was decreased toward normal levels in these growth-retarded animals, arterial and umbilical venous blood PO_2 values, umbilical blood flow, fetal oxygen delivery, and fetal oxygen consumption all increased. The observations from these two growth-retarded fetuses, when combined with the observations we made in normal fetuses, strongly suggest that there is an optimal hematocrit for oxygenation in the fetal lamb, irrespective whether the fetus is normally grown or undergrown in utero.

In response to the question concerning whether the metabolic or hormonal environment of the fetus was affected by our changing the fetal hematocrit, it is likely that the in utero environment of the fetus was altered during our experiments. Nonetheless, the purpose of our studies was to assess the net effect of changes in fetal hematocrit on the intact fetus. To eliminate a potential systematic error due to the direction in which the hematocrit was altered, we increased hematocrit first in half of the fetuses and decreased hematocrit first in the remaining fetuses after determining that there were no differences in the response of fetal oxygenation to alterations in hematocrit relating to the order in which hematocrits were changed (see Methods). Studies were begun only after the fetus was found to be in a stable condition as exemplified by a constant arterial blood pressure, heart rate, and arterial blood gases and pH. In these studies, the mother was unanesthetized and breathing room air.

Concerning the response of hepatic blood flow to changes in fetal hematocrit, we have data relating hepatic blood flow and oxygen delivery to fetal hematocrit. In fetal life, hepatic blood flow is derived from three sources: hepatic arterial blood flow (5% of the total flow), portal venous blood flow (20% of the total), and umbilical blood flow (the remaining 75%). When we increased hematocrit from 10% to about 40% to 45%, blood flow to the liver varied little. At hematocrits >40% to 45%, however, hepatic blood flow decreased substantially. At these high hematocrits, hepatic blood flow decreased for two reasons: (1) total umbilical blood flow decreased at hematocrits above 40% to 45% and (2) the fraction of umbilical blood flow shunted past the liver through the ductus venosus increased. Because of this response of hepatic perfusion to hematocrit, oxygen delivery to the fetal liver was maximal at a hematocrit of 32% and decreased when hematocrits were raised or lowered from that value. This optimal hematocrit for the fetal liver is similar to the optimal hematocrits that we have found for the following organs: gastrointestinal tract organs, spleen, kidneys, placenta, and carcass tissues (skeletal muscle, skin, and bones).

It is generally accepted that the fetus functions in an aerobic environment, even though fetal arterial blood Po_2 is normally low relative to that of the adult. Fetal oxygen consumption in several species, including the human, ranges from 7 to 10 ml of oxygen per minute per kilogram of body weight. This rate is approximately double that of the adult nonpregnant or pregnant animal or human. Despite the greater oxygen consumption rate in the fetus, there is a considerable oxygen reserve, since oxygen delivery to the fetus exceeds oxygen demand by the fetus by about threefold. This oxygen reserve enables the fetus to compensate for variations in its oxygen delivery that may result, for example, from changes in fetal hematocrit or alterations in umbilical or uterine blood flow. Although several fetal responses to stress are similar to those observed in the human, it is quite risky to try to extrapolate results of studies obtained on the lamb fetus to those on the human fetus. In a few situations, extrapolation from experimental data to clinical situations may be warranted, but in many instances this extrapolation may not be valid because of species differences. For example, the basal hematocrit in the human fetus averages 45%, whereas the lamb fetus has a mean hematocrit of about 32%.

In our experiments, oxygen consumption by the fetal lamb was stable over the range of hematocrits from about 16% to 48% but decreased when hematocrits were increased above approximately 48% or decreased below 16% (comparable data for the human fetus are not available). We could not identify the precise hematocrit associated with decreases in oxygen consumption, because curved lines described the relationship of oxygen consumption to fetal hematocrit. We generated these curves with analysis of covariance with which we could take into account individual animal responses. The equations for the curves selected were those having the highest regression coefficients associated with the least residual sums of squares. Tested statistically, these equations best described our data. The observation that oxygen consumption fell at hematocrits above about 48% is not based on data from a single animal. Rather, it is based on the observation that oxygen consumption in several fetuses decreased at relatively high hematocrits.

Fetal oxygen consumption was maintained at a value of about 7 ml of oxygen per minute per kilogram at hematocrits between about 16% and 48% because of alterations in oxygen extraction. Oxygen extraction is that quantity of oxygen consumed by the fetus, expressed as a fraction of the total oxygen delivered to the fetus. Changes in oxygen extraction result from alterations in capillary diffusion variables such as the capillary-to-cell Po_2 gradient or the capillary surface area available for oxygen exchange (capillary density). In fetal life, all organs, with the exception of the heart and the brain, compensate for reductions in oxygen delivery by altering oxygen extraction (in the brain and heart, perfusion increases as compensation, while oxygen extraction does not change substantially). It is not known whether these changes in oxygen extraction in the fetus are primarily due to adjustments in capillary density or to modifications of the capillary-to-cell Po_2 gradient. In the adult animal, adjustments in oxygen extraction occur mainly as a result of increases in capillary density.

Immunotherapeutic studies of different clonal lines of herpesvirus-induced fibrosarcoma in a syngeneic rat

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Transformation of White Buffalo rat embryonic cells by dye-light–inactivated herpesvirus type 2 resulted in the development of two clones of transformed cells (G2 and rat fibrosarcoma) with significantly different tumorigenic capabilities. The G2 cell line was initially nontumorigenic, while the rat fibrosarcoma line was very highly tumorigenic in rats. These significant differences in transformed cell clones from the same initial culture offered an opportunity to study aspects of the immunobiology of oncogenicity. The development of fibrosarcomas in immunoincompetent nude mice with the same early passages of the G2 cell line which were nontumorigenic in the immunocompetent rat suggested that immunologic resistance developed more effectively in the competent host against the G2 line than against rat fibrosarcoma cells. Syngeneic rats which were first exposed to the early passage nontumorigenic G2 cells were completely protected against tumor development by the rat fibrosarcoma cell lines. In subsequent in vitro passages of the G2 cell line, it lost its ability to protect against rat fibrosarcoma challenge and gradually became oncogenic in rats. Modification of antigenic exposure, accomplished by treating the G2 cells with cholesteryl hemisuccinate, resulted in an increase in the protection by these cells and a delay in tumor development. (AM J OBSTET GYNECOL 1985;151:852-8.)

Key words: Immunology, herpesvirus, fibrosarcoma, clone, cell line

In the early 1970s, photodynamic inactivation of recurrent genital herpes infections was considered to be a promising new therapeutic approach.[1-3] These clinical studies were based upon the earlier demonstrations of the inherent photosensitivity of herpesvirus and the significant enhancement of that photosensitivity by heterotricyclic dyes.[4] Although these studies reported favorable clinical results with dye-light treatment, the authors made no mention of other studies which suggested the possibility that this treatment modality could have an oncogenic effect. Duff and Rapp[5] first published evidence that hamster embryo fibroblasts were transformed in vitro after exposure to ultraviolet-irradiated herpesvirus type 2. In a later publication it was shown that photodynamic inactivation of herpesvirus type 1 or type 2 resulted in a virus that could transform hamster embryonic cells.[6] The ability of herpesvirus to transform human cells was documented by Darai and Munk,[7] who reported that human cells showed transformed-like morphologic features following active herpesvirus type 2 infection at 42° C.

In view of these observations, Gusdon and Kucera[8] suggested the oncogenic potential of this treatment modality. Subsequently, photodynamically inactivated herpesvirus type 2 was found to transform human embryonic fibroblasts derived from a therapeutic abortion specimen.[9] The criteria of transformation included: (1) the formation of cell aggregates and the detection of herpesvirus type 2–specific antigen(s) in the abortively infected cells, (2) colony formation in soft methylcellulose overlay medium, and (3) the persistence of viable cells in serial subcultures as compared to the lack of persistence in mock-infected human embryonic fibroblast cell controls.[9]

Oncogenicity by definition requires the development of a malignant tumor upon injection of transformed cells. In order to evaluate the oncogenicity of cells transformed by means of photodynamic inactivation, the same virus and methodology were used to transform rat embryo fibroblasts.[10] The herpesvirus-transformed rat cells were cloned in vitro. Lines were established which varied from nononcogenic (G2) to oncogenic rat fibrosarcoma. The rat fibrosarcoma clonal cell line produced tumors with shorter latent periods (10 to 14 weeks) compared to those of uncloned cells (20 to 24 weeks). After 30 passages in vitro, the rat fibrosarcoma line produced rapidly growing tumors in 100% of the newborn rats with a latent period of only 2 to 3 weeks. Tumor cells (RFS 12-22-75) derived from this highly oncogenic line were reestablished in culture after a single in vivo passage and produced tumors in all cases

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upon subcutaneous inoculation of weanling or adult rats within 2 to 3 weeks. Histopathologic examination of all tumors produced in newborn weanling or adult rats revealed large, poorly differentiated malignant fibrosarcomas, and metastases to the lungs were observed in 20% of newborn animals inoculated subcutaneously with rat fibrosarcoma cells. Further studies by immunofluorescence, with the use of herpesvirus-specific antiserum, showed that 25% to 50% of the clonal transformed or tumor cells synthesized herpesvirus-specific antigens identifiable by surface staining. These cells were resistant to superinfection by the homologous transforming virus.

We therefore have available, from a single parental line, two established clones, one which is nononcogenic and another which was made highly oncogenic by serial in vitro and then a single in vivo passage. These cell lines provided us with a powerful model for investigating the relationship between oncogenicity of a tumor cell and its capacity to induce an immune response. By using the transformed nononcogenic lines as a source of antigen and the oncogenic line to challenge antitumor immunity, it is possible to measure immunity to tumors with both in vitro assays and alteration in the latent period for tumor development (protection). In this study we have made such a comparison in vivo and have found that the nononcogenic clonal line induced immunity to the oncogenic line.

Material and methods

Animals. A colony of syngeneic White Buffalo rats were maintained with careful inbreeding for 8 years by brother-sister matings. Skin transplants were performed intermittently to ascertain that the animals remained syngeneic.

Tissue culture cells and medium. Rat embryo fibroblasts were obtained from 18-day White Buffalo rat embryos derived from the same rat colony as the original culture of transformed cells. The G2 clonal line and rat fibrosarcoma cell lines were derived from the same initial culture previously described.[9] Cell cultures were maintained in Dulbecco's modified Eagle's medium (Grand Island Biological Company, Grand Island, New York) containing mycoplasma-free and virus-screened fetal bovine serum (Flow Labs, Rockville, Maryland). Cells were passaged weekly at the point of confluence into 250 ml tissue culture flasks (Falcon 3024). Aliquots of each passage of the rat fibrosarcoma and G2 cell lines were frozen according to standard methodology, except that we used 10% sterile dimethylsulfoxide in the final resuspension of the cells in Dulbecco's modified Eagle's medium, before freezing in a stepwise fashion.[11] All samples were then stored at −70° C.

Nude mice. In order to determine whether the absence of an immunologic capability could have any effect upon the development of tumor by either G2 or rat fibrosarcoma or rat embryo fibroblast cells, three nude mice each received either 10^4 or 10^6 or 10^7 G2, rat embryo fibroblasts, or rat fibrosarcoma cells.

Tumor dose. To determine the minimum number of cells which invariably resulted in the development of a tumor, 350 4- to 6-month-old male and female White Buffalo rats were injected subcutaneously in the right flank with varying doses of rat fibrosarcoma cells and then observed for 6 months to a year, unless they either died of the tumor, developed intercurrent disease, or were put to death because of the continued growth of the tumor. The cells used were obtained from the culture flasks by trypsinization. They were then washed and counted, and their viability was determined by trypan blue exclusion. Each animal was then injected subcutaneously with the appropriate number of viable cells in 0.5 ml of phosphate-buffered saline solution. All animals were observed three times a week and tumors at the site of injection were measured in millimeters in their greatest diameter and at 90 degrees to that line, in the center of the tumor.

G2 and rat embryo fibroblast cells were studied similarly in a smaller number of animals. Ten animals in each group were each injected subcutaneously with either 10^6, 10^7, or 10^8 G2 cells. Rat embryo fibroblast cells obtained from 18-day midgestation White Buffalo rat embryos were injected into 20 rats at concentrations of either 10^6 or 10^7 cells.

Immunization with the G2 cell line. None of the rats challenged with G2 cells developed tumors, while the rat fibrosarcoma line was invariably tumorigenic. To assess tumor immunity, we challenged a first group of 30 White Buffalo rats that had previously received injections of 10^6, 10^7, or 10^8 G2 cells 6 months earlier and had not developed tumors with 10^6 rat fibrosarcoma cells. Then 30 additional rats injected with the same passages of 10^6 G2 cells were challenged with 10^6 rat fibrosarcoma cells after only 1 month.

In a second experiment, animals were injected with 10^6 G2 cells (passages 31 to 40) and challenged 1 month later with 10^6 rat fibrosarcoma cells. Tumors developed in a number of these animals following rat fibrosarcoma challenge. In a third study, 30 rats were injected with later passages (41 to 50) of 10^6 G2 cells and after 1 month were challenged with 10^6 rat fibrosarcoma cells. Tumors were found to have developed in a large number of these animals before the rat fibrosarcoma challenge. Animals that had not already developed a tumor from the G2 cells were challenged with rat fibrosarcoma cells. Another group of 30 animals were then challenged with 10^7 G2 cells (passages 41 to 50). To enhance the immunogenic stimulus and possibly increase the protection, a fourth experiment was done in which 20

Table I. Titration of tumor development based on the passage of rat fibrosarcoma cells and the number of cells inoculated

	No. of rat fibrosarcoma cells injected subcutaneously				
Passage No.	10^2	10^3	10^4	10^5	10^6
5-20					
No. injected	20	20	20	20	20
No. of tumors	2	4	7	10	20
No. of regressors	1	1	2	2	3
21-30					
No. injected	25	25	25	25	25
No. of tumors	5	9	12	21	25
No. of regressors	2	2	3	3	2
31-50					
No. injected	25	25	25	25	25
No. of tumors	6	12	17	23	25
No. of regressors	0	3	1	2	2

Table II. Tumor development in rats challenged with G2 and rat embryo fibroblasts (initial series).

Experiment No.	*No. of animals*	*Immunogen*	*Passage No.*	*Immunogen-specific tumors*	*No. of rat fibrosarcoma cells injected*	*No. of months after immunogen*	*Rat fibrosarcoma tumors*
1a	10	Rat embryo fibroblasts		0	10^6	6	10
	10	Rat embryo fibroblasts		0	10^7	6	10
	10	G2	5-10	0	10^6	6	0
	10	G2	11-20	0	10^7	6	0
	10	G2	21-30	0	10^8	6	0
1b	30	G2	10-30	0	10^6	1	0
2	30	G2	31-40	0	10^6	1	4
3	30	G2	41-50	7	10^6	1	0*
	30	G2	41-50	12	10^7	1	0*
4	20	G2	51-90	12	$10^6 \times 2$†	1	0*
5	20	G2	51-90	10	10^6	1	6

*Animals which did not develop a tumor upon injection of G2 cells were protected against the subsequent development of tumor by the rat fibrosarcoma cells.

†Weekly injections two times.

animals were injected with 10^6 G2 cells (passages 51 to 90) twice, with a 1-week interval. This experiment was repeated with only a single injection of G2 cells. In every experiment in which rat fibrosarcoma cells were used to challenge G2-exposed animals, 10 control animals were injected with 10^6 rat fibrosarcoma cells from the same cultures to ascertain that no defect or change in the tumorigenicity of the rat fibrosarcoma cells had occurred.

After it was noted that the experiments differed principally in the passage number of the cell line, different passages of the G2 cell line were brought into culture, and 240 animals were injected with 10^6 G2 cells from the different passages.

Cell membrane modification and immunization. With the use of a slight modification of the method of Shinitzky et al.,[12] both G2 and rat fibrosarcoma cell membranes from different passages were treated with cholesteryl hemisuccinate (Sigma Chemical Co., St. Louis, Missouri). The 3.5% w/v polyvinylpyrrolidone was made up in a solution of RPMI medium. Then 10^6 cells (either G2 or RFS) were incubated at room temperature for 2.5 to 3.0 hours with gentle shaking and then washed twice with RPMI medium and resuspended in RPMI medium without serum at a concentration of 2×10^6/ml, to be used for injection.

The animals were injected subcutaneously with either 10^6 cholesteryl hemisuccinate–modified G2 cells or 10^6 cholesteryl hemisuccinate–modified rat fibrosarcoma cells. One month later, all animals that had not developed tumors were injected with 10^6 rat fibrosarcoma cells.

Results

The number of cells required to produce tumors as a function of the in vitro passage are shown in Table I. High-passage rat fibrosarcoma cells were found to be more tumorigenic than the lower-passage cells; the

Table III. Analysis of protection by different passages of G2 cell line against fibrosarcoma (repeat series)

No. of animals tested	*Passage of G2 cells*	*Immunogen-specific tumors*		*Rat fibrosarcoma tumors*		*Protection (%)*
		No.	*%*	*No.*	*%*	
40	5-10	0	0	0	0	100
40	11-20	0	0	0	0	100
40	21-30	0	0	0	0	100
40	31-40	2	5	5	18	87
40	41-50	5	13	10	29	71
40	51-90	20	50	20	100	0

50% oncogenic dose varied from 10^5 cells (passages 5 to 20) to 10^3 cells (passages 31 to 50). Injection of one million rat fibrosarcoma cells invariably resulted in the development of a tumor detectable 14 days after inoculation that grew to an average size of approximately 200 mm^2 30 days after inoculation. Lower doses of rat fibrosarcoma cells resulted in a longer latent period prior to development of an obvious tumor. Approximately 10% of all tumors that developed to 200 mm^2 regressed by 60 days after inoculation, and scar tissue was apparent. With as few as 100 cells injected, some animals developed progressive tumors from each passage series.

Tumor development from rat fibrosarcoma cells in White Buffalo rats previously injected with either G2 or rat embryo fibroblasts cells is shown in Table II. Ten animals were each injected subcutaneously with either 10^6, 10^7, or 10^8 G2 cells from passages 5 to 30, without any tumor development noted over a 6-month period of time. After 6 months of observation without any tumor development, all of these animals were challenged with 10^6 rat fibrosarcoma cells, and none of them developed tumors. In this and all subsequent studies in which G2-immunized animals were challenged with rat fibrosarcoma cells, aliquots of the same culture and passage of the rat fibrosarcoma cells (10^6) were injected into 10 nonimmunized rats. All control animals in this and subsequent experiments developed tumors. To determine if protection could be achieved by immunization in a shorter period of time, 30 animals that received the same passage levels of 10^6 G2 cells were challenged with 10^6 rat fibrosarcoma cells after only 1 month. The protection against tumor development was complete (experiment 1b, Table II). Subsequent experiments were therefore done with only a 1-month interval between immunization and challenge

To test the hypothesis that immunologic resistance was involved in the failure to develop tumors with the G2 cell line, nude mice, which are immunologically incompetent, were injected with either rat embryo fibroblast or G2 (passages 10 to 25) or rat fibrosarcoma cells. None injected with rat embryo fibroblast cells developed tumors. Those injected with rat fibrosarcoma cells developed rapidly growing tumors detectable in less than a week. Within 2 to 3 weeks after subcutaneous G2 challenge, every nude mouse had a detectable tumor. In the mice given 10^7 G2 cells, the tumor was detectable within 2 weeks. Once these tumors started to grow, they advanced as rapidly as the rat fibrosarcoma tumors. The histopathologic features of the fibrosarcomas that developed in the G2-injected nude mice and the rat fibrosarcoma–injected animals were identical.

None of the rats that had received either G2 or rat embryo fibroblast cells in the first experiment developed any tumors prior to challenge with the rat fibrosarcoma cell line. As shown in experiment 1 (Table II), all animals immunized with the G2 line were resistant to tumor development upon challenge with the rat fibrosarcoma line and no animals immunized with the untransformed rat embryo fibroblasts cells were resistant.

In subsequent experiments (2 to 5), the clear distinction between the nononcogenic but protective G2 line and the oncogenic rat fibrosarcoma line was not apparent. In experiment 2, none of the G2-immunized animals developed tumors from the G2 cells, but four of 30 subsequently challenged with rat fibrosarcoma cells did. In experiments 3 to 5, the immunogen itself was oncogenic in seven of 30 (experiment 3) to 12 of 20 (experiment 4) animals. Animals not developing tumors as a result of injection of the "immunogen" were resistant to subsequent challenge with rat fibrosarcoma cells. The tumors that developed after "immunization" with the G2 line and without injection of the rat fibrosarcoma line were histopathologically indistinguishable from those produced by the rat fibrosarcoma line alone.

Upon careful examination of the characteristics of the G2 immunogen, it became apparent that the strongly protective cells (experiment 1) were from early passages (5 to 30) and the weakly protective and tumorigenic cells (experiments 3 to 5) were from later passages (30+) of the cell line. This was consistent with the increased oncogenicity of the rat fibrosarcoma line upon in vitro passage[10] and suggested that similar changes had occurred in the G2 line. To test this hy-

Table IV. Protection against rat fibrosarcoma tumor challenge by cholesteryl hemisuccinate–modified G2 cells

Passage	*No. of animals*	*No. of G2 tumors*	*No. of tumors on rat fibrosarcoma challenge*	*Protection (%)*
10-20	20	0	0	100
21-30	20	0	0	100
31-40	20	0	0	100
41-50	20	0	0	100
51-90	20	12	6	25

pothesis, the effect of passage number on the oncogenicity of the G2 line and the protection from subsequent rat fibrosarcoma challenge 1 month later was studied. The results of these experiments are presented in Table III. Tumors developing during the period of observation prior to the injection of rat fibrosarcoma cells into the G2-immunized rats are designated as immunogen-specific tumors. Tumors that developed subsequent to challenge with the rat fibrosarcoma cells were designated as rat fibrosarcoma tumors.

Through passage 30, the protection conferred by the G2 cell line was complete, but thereafter the degree of protection from rat fibrosarcoma tumor development decreased significantly, and the oncogenicity of the G2 cell line increased as the passage level increased. The tumors that developed as a result of G2 cell injection were examined microscopically, and all were fibrosarcomas indistinguishable from those formed after the injection of the rat fibrosarcoma cell line. The antigenic (G2) and oncogenic (rat fibrosarcoma) cell lines are readily distinguishable by their morphologic features in culture.[10] Careful analysis of cultures indicated that there had not been any cross contamination of the two cell lines used in these experiments.

To determine if modification of cell membrane fluidity, with the achievement of an increased antigenic presentation to the host, would have any effect upon protection against the development of tumors, the different passages of the G2 cell line were treated with cholesteryl hemisuccinate, since Shinitzky et al.[12] had demonstrated an increased immunity in animals to tumors so treated. The results of these studies are presented in Table IV. Up to passage 50, cholesteryl hemisuccinate–modified G2 cells completely protected against tumor development with the rat fibrosarcoma line. This protective capacity had been reduced in passages beyond 30 in the untreated G2 cells (Table II). Beyond passage 50, the increased protection afforded by cholesteryl hemisuccinate treatment was not sufficient to prevent tumor development from the cholesteryl hemisuccinate–modified G2 line alone. Modification of the rat fibrosarcoma cells by cholesteryl hemisuccinate had no effect upon the development of tumors.

Comment

In this study, we had the opportunity to compare the immunogenic and oncogenic characteristics of two transformed clonal cell lines, derived from the same original culture of White Buffalo rat embryonic fibroblasts infected with dye-light–photoinactivated herpesvirus type 2. The cell line designated rat fibrosarcoma was found to be highly tumorigenic and became increasingly malignant with continued in vitro passage.

The G2 cell line, although having all of the in vitro characteristics of transformation, did not result in the development of any tumors when injected into syngeneic rats. These studies were undertaken in an effort to exploit this significant difference in tumorigenicity, with the hope that we might be better able to understand oncogenicity.

Studies were undertaken to determine whether the differences in tumorigenicity of these two cell lines might reside in a more effective immunologic recognition and host reaction to the G2 cell line. Our results strongly suggest that G2 and rat fibrosarcoma cells carried cross-reactive antigens, since exposure to the G2 cells protected animals from a subsequent challenge with a 100% tumorigenic dose of rat fibrosarcoma cells. The G2 cell line itself was tumorigenic even in low-passage numbers, as seen by the development of tumors in an immunoincompetent host, the nude mouse. These were the same G2 cells which, when injected into the syngeneic strain immunocompetent rat host, resulted not only in a lack of tumor development but in subsequent protection against tumor by the rat fibrosarcoma cell line.

The loss of the protective capability and the increasing tumorigenicity of the G2 cell line were puzzling. Having ascertained that no cross contamination of the two cell lines had occurred, we were able to relate these changes to continued passage of the cells in vitro. Repetition of studies with all passages of the G2 cells which had been preserved in a frozen state was accomplished, and it was verified that the decrease in protective capability and the increased tumorigenicity of the G2 cells were a function of the increased passage in culture. There was a selection of cells with increased tumorigenicity by serial passage.

We hypothesized that the difference between the cell lines might be the result of a difference in relevant antigenic presentation. It is now well accepted that many tumor cells bear antigenic determinants that may elicit an immune response.[13-15] The development of a tumor is a function of both the rate of cellular division and the opposing rate of tumor rejection. Tumors that have the greatest chance to develop would be those that stimulate the least immune resistance. When the rate of rejection exceeds the rate of multiplication, either tumors do not develop or they regress. Although many factors could affect this situation, the degree of presentation of tumor-associated antigens could be a highly significant factor.

Borochov and Shinitzky,[16] Shinitzky and Rivnay,[17] and Borochov et al. demonstrated that increasing membrane-lipid microviscosity by the incorporation of cholesterol resulted in an increase in the exposure of membrane proteins, while depletion of cholesterol had the converse effect.[16-18] Augmentation of tumor immunogenicity by more effective exposition of the tumor-associated antigens was then accomplished by increasing the microviscosity of tumor cell membranes.[12]

Our system offered an opportunity to study whether increasing the microviscosity of the G2 cell membranes, as they increased in cell passage and lost their protective ability, would have any effect upon their protective capacity. A recovery of protection was achieved, as evidenced by an increased protection rate with increasing passage of the G2 cells and the absence of development of immunogen-specific tumors until beyond passage 50. G2 tumors had occurred in previous studies as early as passage 40.

Directions for further study of this interesting model system include: (1) a study of the microviscosity of the G2 cell membranes as a function of cell passage level in vitro, (2) the isolation and characterization of the tumor-associated antigens, and (3) the development of monoclonal antibodies against those antigens. This model system should permit us to determine what properties of cells (physical, antigenic expression) are responsible for tumorigenicity and what properties induce tumor immunity. This information may be valuable in designing rational protocols for immunotherapy.

We acknowledge the technical assistance of Glenn A. Herbst, M.A. This work is dedicated to the memory of Marcelle Deiber Gusdon.

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Discussion

DR. JAMES R. SCOTT, Salt Lake City, Utah. This study is a sequel to one in which Dr. Gusdon showed that photoinactivated herpes simplex virus had a carcinogenic potential. Two cell lines isolated had in vitro characteristics of transformation but one clone (G2) was a regressor when injected into animals and one clone (rat fibrosarcoma) a progressor. While the in vivo behavior of these two clones was very dissimilar, antigen sharing was apparent since prior immunization with the G2 clone protected against tumor formation when the immunized animals were challenged with the progressor clone rat fibrosarcoma. A major point addressed by the paper is the fact that G2 developed progressor characteristics after serial in vitro passages. However, the isolation of both progressor and regressor clones from the same tumor and the change in characteristics after serial in vitro passages are not uncommon phenomena. In our own laboratory, Daynes and Beeson have produced similar results using ultraviolet light radiation and placental cell culture systems. Does Dr. Gusdon believe that simple selection or actual modulation or transformation of the cell line is taking place?

Perhaps of greater potential significance is the fact that treatment of G2 cells with cholesteryl hemisuccinate reduced the tumorigenicity of the high-passage G2 line and restored some of its ability to protect against rat fibrosarcoma, presumably through alterations in cell membrane microviscosity. This finding could have important implications in tumor immunotherapy. However, the microviscosity assays needed to prove the induction of membrane modifications were not conducted. Treatment of cells with cholesterol, depending on the purity of the reagents, can mediate a large number of potential changes of the treated cells, and microviscosity represents only one of these. Does Dr. Gusdon have any further data to clarify this point?

In conclusion, I congratulate Dr. Gusdon and encourage him to pursue the lines of research he has suggested. To some extent, tumor immunology is a murky area that has had little impact on the clinical management of cancer. Nevertheless, there was a time when many infectious diseases that are now easily cured seemed almost as inevitable in their course as cancer does today. In depth studies at the molecular level in virology, immunology, and biochemistry are urgently needed if we are eventually to understand the development and control of human neoplasms.

Dr. Roland A. Pattillo, Milwaukee, Wisconsin. Dr. Gusdon, the work by Gey a number of years ago showed spontaneous transformation of the rat fibroblast in culture when maintained for prolonged passages.

The finite life expectancy of cells in culture has been suggested to be in the range of approximately 50 passages, after when spontaneous transformation takes place and these cells are then capable of inducing tumors.

My question is have you actually demonstrated any effect of fluorescent light and dye–treated rat embryo fibroblast or are you actually seeing spontaneous transformation of these cells with the production of tumors that have nothing to do with the dye or fluorescent light treatment?

To answer that question, did you specifically study parallel rat embryo fibroblasts that were not treated by dye or fluorescent light?

Dr. Seymour L. Romney, New York, New York. Dr. Gusdon, is your system essentially only proving that tumors are heterogeneous, which you have succeeded in demonstrating in a variety of passages?

Second, have you considered using retinoids to determine their protective, possibly preventive action?

Finally, when you began working with herpesvirus, was it because you thought it might be responsible for cervical cancer?

Dr. Gusdon (Closing). Dr. Scott, I do not know whether there is simple selection by the pressures of continuous passage or if there is some internal modulation. I cannot answer that.

With regard to your question about whether microviscosity studies have been done along the different passages, that was part of the next grant request. However, in order to make that grant request, we did probe and study the very early passages along with the very late passages and did find a very significant change. In answer to your question, this has never been done in humans.

With regard to monoclonal antibodies, we have somewhere in the neighborhood of 30 of these already manufactured.

Dr. Pattillo, we did continue passages of rat embryo fibroblasts in culture. We did expose them to dye and light. There was no transformation, and in our hands, after approximately 12 passages, untransformed rat embryo fibroblasts tend to wither away.

Dr. Romney, tumors are heterogeneous. I have no response to that. We have never used retinoids in this system, and I have no plan to study that aspect.

We began the study of herpesvirus early in the 1970s simply because I have a joint appointment in the Department of Microbiology. I was working with virologists, and I suspected at that time that herpesvirus was very dangerous. I think that was engendered from long ago because of the relationship to cervical cancer.

Effects of maternal ethanol infusion on fetal cardiovascular and brain activity in lambs

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Ethanol (1 gm/kg of maternal body weight administered over 1 hour) was infused intravenously into 11 chronically prepared pregnant ewes between 128 to 137 days' gestation. Fetal breathing movements were suppressed for 9 hours following ethanol administration, and both high- and low-voltage fetal electrocortical activity were suppressed for 3 hours and replaced by intermediate-voltage electrocortical activity. Fetal blood gases and pH were not altered. These data support the hypothesis that ethanol suppresses fetal breathing movements by a direct central mechanism rather than indirectly by alteration of electrocortical activity. (AM J OBSTET GYNECOL 1985;151:859-67.)

Key words: Ethanol, fetal brain activity, fetal cardiovascular function

Chronic alcohol abuse during pregnancy has been associated with the fetal alcohol syndrome.[1,2] This syndrome, which involves craniofacial abnormalities, prenatal and postnatal growth deficiencies, and mental retardation, represents the tip of a spectrum of possible effects of alcohol on the fetus. Although babies with fetal alcohol syndrome are increasingly recognized as a complication of heavy alcohol consumption during pregnancy, recent data suggest that newborn infants may have less obvious effects from moderate maternal ethanol consumption during pregnancy. Streissguth et al.[3] reported that women who were not alcoholics but who consumed moderate amounts of alcohol during pregnancy had a higher incidence of babies with respiratory distress at birth, lower Apgar scores, bradycardia, and variable decelerations during labor.

We extended the original observations of Fox et al.[4] and reported that a small dose of ethanol (0.25 gm/kg) administered orally to pregnant women near term almost abolished fetal breathing activity within 30 minutes and suppressed fetal breathing activity for at least 3 hours following ethanol administration.[5] Further, we recently reported that maternal intravenous infusion of glucose at 30 minutes following maternal ethanol consumption produced no significant alteration of the suppressed fetal breathing activity[6] whereas maternal glucose administration alone has been demonstrated to increase the incidence of fetal breathing movements.[7]

To directly examine effects of ethanol on fetal cardiovascular and brain function, we developed a chronic fetal lamb model. Preliminary studies were performed to determine a dose and method of administration which reproduced the effects of ethanol on fetal breathing movements, originally reported in women. In the current study the effects of a maternal infusion of ethanol, 1 gm/kg of maternal body weight given over 1 hour, on fetal lamb cardiovascular and brain activity were determined.

Material and methods

Studies were performed on 12 fetal lambs of mixed breed between 128 and 137 days' gestation (term, 147 days). Fetuses were surgically prepared at least 4 days prior to the beginning of experiments. Ewes were anesthetized with 40 mg of thiopental sodium intravenously, intubated, and maintained on closed circuit ventilation with 1.5% to 2.0% halothane and a 50:50 mixture of nitrous oxide and oxygen at 1 L/min.

Polyvinyl catheters were placed in the brachiocephalic trunk via a fetal forelimb artery for sampling preductal arterial blood, in the inferior vena cava via a fetal pedal vein, and in the sagittal sinus as previously reported.[8] Tracheal and amniotic fluid catheters were also placed as has been previously described.[9] Electrodes of stainless steel Teflon-coated wire (Cooner Corporation, Chatsworth, California) were implanted biparietally on the dura for fetal electrocortical activity, through the orbital ridge of the zygomatic bone of each eye for electroocular recordings, and over the sternum for fetal heart rate recordings. A reference electrode

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was placed in the loose connective tissue overlying the occipital bone of the skull. The catheters were filled with a heparinized saline solution; catheters and wires were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters were then placed in the maternal femoral artery and vein. At the time of surgery, 1 gm of dihydrostreptomycin and 800,000 U of penicillin G were injected intramuscularly into the ewe, and after the uterus was closed, 1,000,000 U of penicillin G was injected into both the fetal hind limb vein and the amniotic cavity. The antibiotic coverage was continued daily for 2 to 3 days. After the operation animals were maintained in individual cages suitable for continuous monitoring, with food and water available.

Physiologic measurements

Each fetal lamb was studied during a fed control condition, a subsequent period of ethanol exposure, and a postethanol recovery period. Strain gauge manometers (Statham Model P-23ID, Gould Inc., Oxnard, California) and a chart recorder (Model 7, Grass Instrument Co., Quincy, Massachusetts) were used to record pressure in the trachea, amniotic fluid, and brachiocephalic artery. Mean arterial blood pressure was calculated as diastolic plus 0.4 of the difference of the systolic minus diastolic pressure. Electrical signals were processed by means of a common mode rejection preamplifier (Model 7, P511J, Grass Instrument Co., Quincy, Massachusetts). Fetal heart rate was measured by means of a cardiotachometer (Model 7, P44B, Grass Instrument Co., Quincy, Massachusetts). Fetal electrocortical and electroocular potentials were displayed directly on the chart recorder after passage through a passive band-pass filter, 0.3 to 30 Hz on the preamplifier.

Continuous recordings of electrocortical activity, electroocular recordings, tracheal pressure, amniotic pressure, fetal arterial blood pressure, and fetal heart rate were commenced 24 hours before each ethanol infusion. A 40% (v/v) solution of ethanol (Alcohol, Liquor Control Board of Ontario) was administered by Harvard infusion pump (Harvard Apparatus, Dover, Massachusetts) into the maternal inferior vena cava for 1 hour at a dose of 1 gm/kg of maternal body weight/hr (n = 11). Continuous recordings were made for a further 24 hours following infusion. Approximately 30 minutes before initiation of the ethanol infusion, fetal and maternal arterial blood samples were taken. After initiation of the infusion, fetal and maternal arterial blood samples and an amniotic fluid sample were taken every 15 minutes for the first hour, every half hour for the next 3 hours, and hourly for the next 6 hours (n = 11) or 10 hours (n = 4). All samples were analyzed for ethanol concentrations (0.25 ml sample). Fetal arterial blood samples taken during the control period and at 1 and 2 hours were also analyzed for blood gases, pH, and whole blood glucose (0.8 ml sample).

Approximately 30 minutes before ethanol infusion, radionuclide-labeled microspheres were injected into the fetal inferior vena cava for determination of brain and myocardial blood flow, followed immediately by a fetal blood sampling from the brachial artery and sagittal sinus. At the end of the ethanol infusion and again 1 hour after infusion, second and third microsphere injections were performed together with paired fetal blood sampling. The results of these experiments are reported separately.[10]

Fetal blood pH, P_{O_2}, and P_{CO_2} were analyzed with an ABL3 blood gas analyzer with temperature corrected to 39° C (Radiometer Ltd., Copenhagen, Denmark). Glucose in whole blood was measured in triplicate with a membrane bound enzyme technique utilizing the glucose oxidase method (Model 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio).

Duplicate 0.1 ml fetal arterial blood samples were analyzed quantitatively for ethanol. Each 0.1 ml sample was immediately placed in an ice-cold 0.9 ml solution that contained 0.865 ml of a saline solution of 34 mg/ml of perchloric acid and 65 μg/ml of sodium azide, 0.01 ml of an aqueous solution of 76 mg/ml of thiourea, and 0.025 ml of an aqueous solution of 3 mg/ml of 1-propanol. After a 5-second mixing, the sample was centrifuged at 13,000 × g for 30 seconds in a microcentrifuge. A 0.2 ml aliquot of the supernatant was placed in a 1.5 ml glass vial, which then was sealed with a silicon septum, and frozen on dry ice until analyzed within 36 hours. The ethanol concentration was determined by gas-liquid chromatography with use of headspace analysis as previously described.[11, 12] The accuracy of the procedure for ethanol is 101.9% ± 0.7% (SEM), the coefficient of variation is less than 2.5%, and the lower limit of quantitative sensitivity is 0.005 mg/ml.

Data analysis

The zero-order ethanol elimination rate (mg/ml/hr) from fetal arterial blood was determined from the apparent linear portion of the elimination phase of the ethanol concentration–time curve of the individual animals by the use of linear regression analysis.[13]

Electrocorticol activity was assessed by visual analysis into periods of high-voltage (100 to 200 μV), low-voltage (<50 μV), and intermediate-voltage (50 to 100 μV) activity (n = 11). The incidence of electroocular activity was also quantified (n = 11). The incidence of fetal breathing movements was determined by negative pressure deflections of the tracheal pressure recordings and reported as the percentage of time in each hour spent breathing. Fetal heart rate and blood pressure

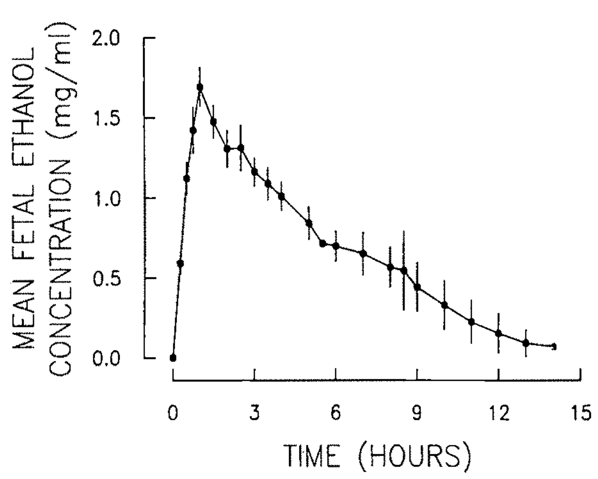

Fig 1. Mean (±SEM) fetal arterial blood ethanol concentration–time curve for 1-hour maternal intravenous infusion of ethanol, 1 gm/kg of maternal body weight.

recordings were analyzed every 5 minutes and reported as average values for each hour (n = 11).

Results are reported as the means and standard error of the means. Statistical significance was calculated with a *t* test for small samples with variance equal, as verified by an F test described by Bailey.[14]

Fetal ethanol concentrations

Fetal arterial blood ethanol concentrations reached a peak of 1.69 ± 0.13 mg/ml at 1 hour (end of maternal ethanol infusion) and decreased to 0.70 ± 0.10 mg/ml at 6 hours and 0.07 ± 0.08 mg/ml at 14 hours following the initiation of ethanol infusion (Fig. 1). The rate of ethanol elimination from fetal arterial blood was 0.14 ± 0.01 mg/ml/hr.

Fetal breathing and cardiovascular measurements

The incidence of fetal breathing movements was 35.4% ± 1.3% for the 8 hours before ethanol infusion and decreased to 10.3% ± 1.1% for the 5 hours following initiation of ethanol infusion ($p < 0.001$). Breathing movements increased slightly to 21.6% ± 1.4% from the sixth to ninth hour ($p < 0.001$ compared to first 5 hours) and returned to an incidence of 34.0% ± 0.5% from the tenth to twelfth hour, which was not significantly different from the incidence before ethanol infusion (Fig. 2).

The fetal heart rate increased from 162 ± 1 bpm during the 4 hours before ethanol infusion to 168 ± 1 bpm during the 4 hours after the onset of ethanol infusion ($p < 0.05$, paired *t* test) and to 167 ± 1 bpm from the fifth to eighth hour (NS) (Fig. 3). Fetal mean arterial blood pressure was 42.5 ± 3.4 mm Hg before infusion and did not change significantly following ethanol administration.

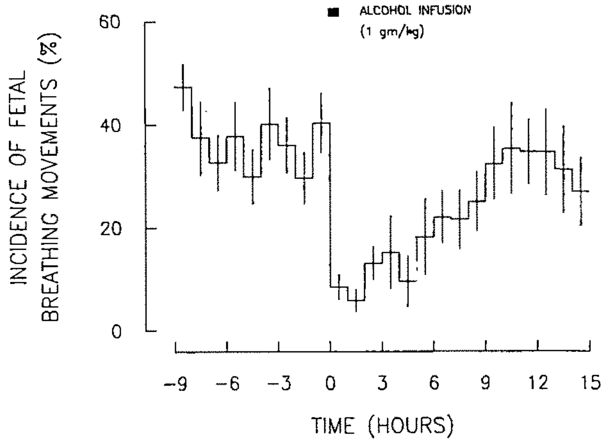

Fig. 2. The incidence of fetal breathing movements per hour (±SEM) was plotted before and after alcohol infusion. There was a significant decrease in the incidence of fetal breathing movements for 9 hours following the onset of the 1-hour ethanol infusions.

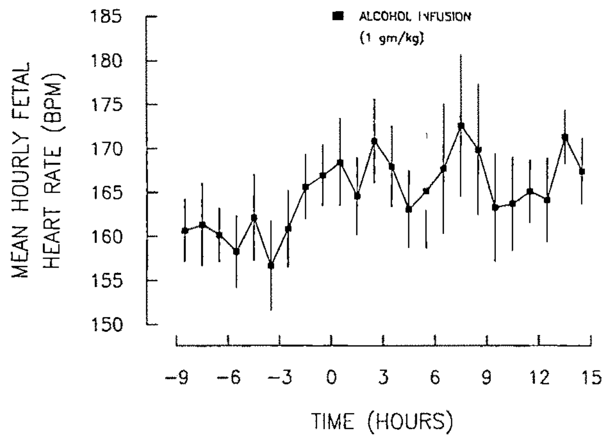

Fig. 3. The mean hourly fetal heart rate increased significantly for 4 hours after the onset of ethanol infusion.

Fetal electrocortical and electroocular activity

The mean incidence of low-voltage electrocortical activity was 48.1% ± 1.5% for the 8 hours before ethanol and fell to 22.7% ± 3.0% for the 3 hours after initiation of ethanol infusion ($p < 0.001$) and returned to 46.2% ± 1.2% from the fourth to the eighth hour (NS compared to control). The mean hourly incidence of high-voltage electrocortical activity also decreased from a mean of 39.8% ± 1.1% for 8 hours before ethanol to 21.8% ± 2.0% for the 3 hours following initiation of ethanol infusion ($p < 0.001$) and returned to 36.6% ± 1.1% from the fourth to eighth hour (NS compared to control) (Fig. 4).

Electroocular activity occurred almost exclusively during low-voltage electrocortical activity (Fig. 5) and was present 86.0% ± 1.6% of the time during this state with little change after ethanol administration. Overall,

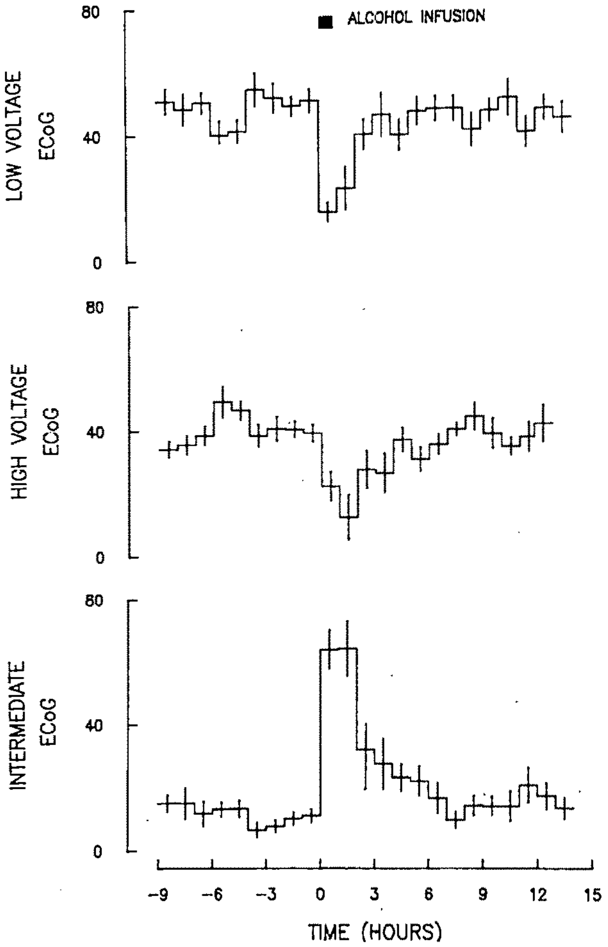

Fig. 4. The incidence of high-voltage (100 to 200 μV), low-voltage (<50 μV), and intermediate-voltage (50 to 100 μV) electrocortical activity was plotted each hour (±SEM) before and after alcohol infusion.

the mean incidence of electroocular activity 8 hours before ethanol administration was 51.2% ± 1.6% and fell to 31.2% ± 1.6% during the 3 hours after initiation of ethanol infusion ($p < 0.005$, paired *t* test) and returned to 47.6% ± 1.3% from the fourth to eighth hour (NS compared to control) (Fig. 6).

During the first 3 hours following onset of ethanol infusion an electrocortical activity pattern of intermediate voltage (50 to 100 μV) with sporadic electroocular activity occurred 55.7% ± 3.7% of the time, which was significantly different from the pattern 8 hours before ethanol infusion, or the fourth to eighth hour following initiation of ethanol infusion (12.0% ± 0.7% and 17.3% ± 1.7%, respectively; both $p < 0.001$). (Fig. 4).

Fetal arterial blood gases and glucose concentration

Fetal arterial Po_2, Pco_2, and pH were 20.6 ± 1.3 mm Hg, 46.4 ± 0.8 mm Hg, and 7.35 ± 0.01 and did not change at 1 or 2 hours following initiation of ethanol infusion (Table I). There was a decrease in the fetal arterial whole blood glucose concentrations from 19.3 ± 1.2 mg/dl before infusion to 16.0 ± 1.2 and 15.9 ± 1.4 mg/dl at 1 and 2 hours following initiation of ethanol administration (both $p < 0.001$, paired *t* test).

Other observations

During the experiments ewes did not exhibit unusual agitation; however, for the first 2 to 3 hours following ethanol administration, sheep appeared to have impairment of balance and spent a greater part of the time lying down and did not eat or drink. They returned to regular activity within 3 hours of the onset of infusion with no apparent ill effect.

In one control animal the same experimental protocol was used except that an equal volume of normal saline solution was infused intravenously over 1 hour to the ewe. In this animal the following average measurements were obtained and did not change from 8 hours before to 8 hours after saline solution administration: fetal breathing movements, 48.2% ± 8%; low-voltage electrocortical activity, 49.9% ± 7.1%; high-voltage electrocortical activity, 47.8% ± 6.3%; electroocular activity, 51.5% ± 6.7%; mean arterial blood pressure, 42.9 mm Hg; mean fetal heart rate, 165 bpm; Po_2, 18.6 ± 0.7 mm Hg; Pco_2, 51.6 ± 2.3 mm Hg; pH, 7.31 = 0.01; glucose, 19.0 ± 0.1 mg/dl.

Comment

In this study, administration of 1 gm/kg of ethanol over 1 hour into the maternal inferior vena cava resulted in an abrupt decrease in the incidence of low- and high-voltage electrocortical activity within 30 minutes after the onset of ethanol infusion, which lasted for about 3 hours before returning to baseline values. Similar observations were made for the incidence of electroocular activity that represents rapid eye movements, which were usually present during low-voltage electrocortical activity. Electroocular activity continued to be present the same proportion of the time during episodes of low-voltage electrocortical activity before or after ethanol administration (86%). During the 3 hours following the onset of ethanol administration, the typical electrocortical patterns were replaced with intermediate-voltage electrocortical activity (50 to 100 μV) with occasional sporadic electroocular activity. Mann et al.[15] demonstrated a similar change in electrocortical activity in studies on acute fetal lamb preparations after ethanol administration.

Fetal breathing movements occur about 30% to 40% of the time in association with low-voltage electrocortical activity and rapid eye movements. The incidence of fetal breathing activity decreased within 30 minutes of the onset of ethanol infusion and remained signifi-

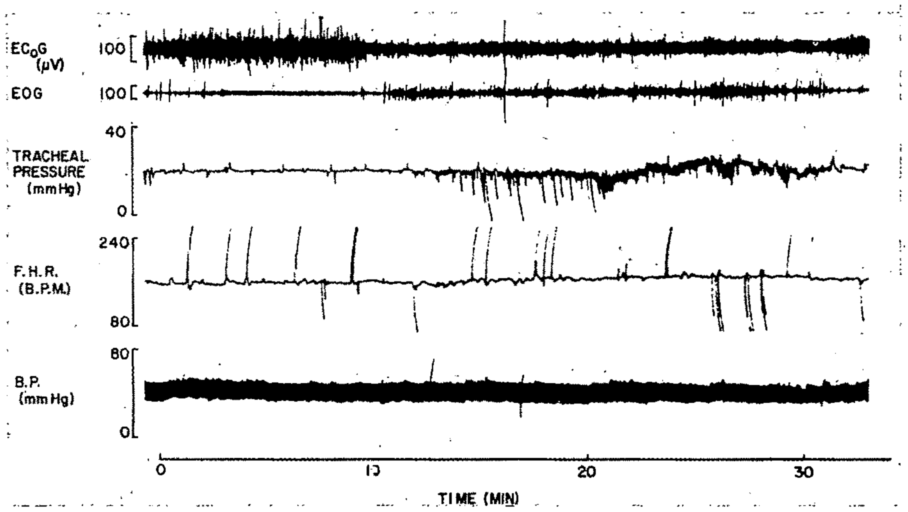

Fig. 5. Chart recording demonstrating that electroocular activity *(EOG)* and fetal breathing movements normally occur during times of low-voltage electrocortical activity. *ECoG,* electrocortical activity; *FHR,* fetal heart rate; *BP,* blood pressure.

Table I. Effect of ethanol on fetal arterial blood gases and glucose concentrations

	Control	*1 hour after initiation of ethanol*	*2 hours after initiation of ethanol*
Po_2 (mm Hg)	20.6 ± 1.3	22.1 ± 1.1	22.4 ± 1.1
Pco_2 (mm Hg)	46.4 ± 0.8	45.9 ± 0.6	43.8 ± 0.8
pH	7.35 ± 0.01	7.35 ± 0.01	7.36 ± 0.01
Glucose (mg/dl)	19.3 ± 1.2	16.0 ± 1.2*	15.9 ± 1.4*

*$p < 0.001$ when compared to control (paired *t* test).

cantly depressed for 9 hours, by which time the fetal arterial blood ethanol concentration had decreased to 0.44 ± 0.18 mg/ml. In this study, the incidence of low-voltage electrocortical activity and electroocular activity returned to normal 3 hours after initiation of ethanol administration, but it was 6 hours longer before the incidence of breathing activity returned to baseline levels (Fig. 6). Therefore the suppression of fetal breathing movements by maternal ethanol infusions could not be explained by changes in electrocortical activity. In fetal lambs, Dawes et al.[16] performed caudal brain stem sections at the level of the upper pons and observed that episodic fetal breathing movements and electrocortical activity became dissociated following this procedure. Furthermore, they measured persistence or enhancement of fetal breathing activity during isocapnic hypoxia, which normally completely suppresses breathing activity in the intact fetal lamb. In three fetuses with brain stem section, Dawes measured almost continuous fetal breathing movements before a maternal infusion of 60 ml of absolute ethanol, but following the infusion, fetal breathing activity disappeared for 0.5 to 4.5 hours (Dawes, personal communication). He concluded that ethanol can have a direct effect on brain stem centers below the pons which are concerned with control of fetal breathing activity. This effect contrasts with that of pentobarbital, which caused prolonged high-voltage electrocortical activity and arrest of fetal breathing movements by wholly suprapontine mechanisms.

Recently Mukherjee and Hodgen[17] reported that maternal alcohol administration to pregnant rhesus and cynomolgus monkeys resulted in impairment of the umbilical circulation, fetal hypoxia, and asphyxia. However, these investigators injected a very high dose of ethanol over 1 to 2 minutes (3 gm/kg) and were, in fact, using near lethal doses of ethanol. In the current study we were unable to demonstrate any changes in fetal arterial pH, Po_2, or Pco_2, and so it would appear that factors other than changes in fetal blood gases are involved in the diminished fetal breathing activity following ethanol administration.

There was a small decrease in fetal arterial whole blood glucose concentrations at 1 and 2 hours following ethanol administration. It has been demonstrated in chronic fetal lamb preparations that the incidence of fetal breathing activity is reduced when the fetal blood glucose concentration is 10 mg/dl and that breathing activity is arrested when the fetal blood glucose concentration falls below 8 mg/dl. The fetal blood glucose concentration in our study did not decrease far enough

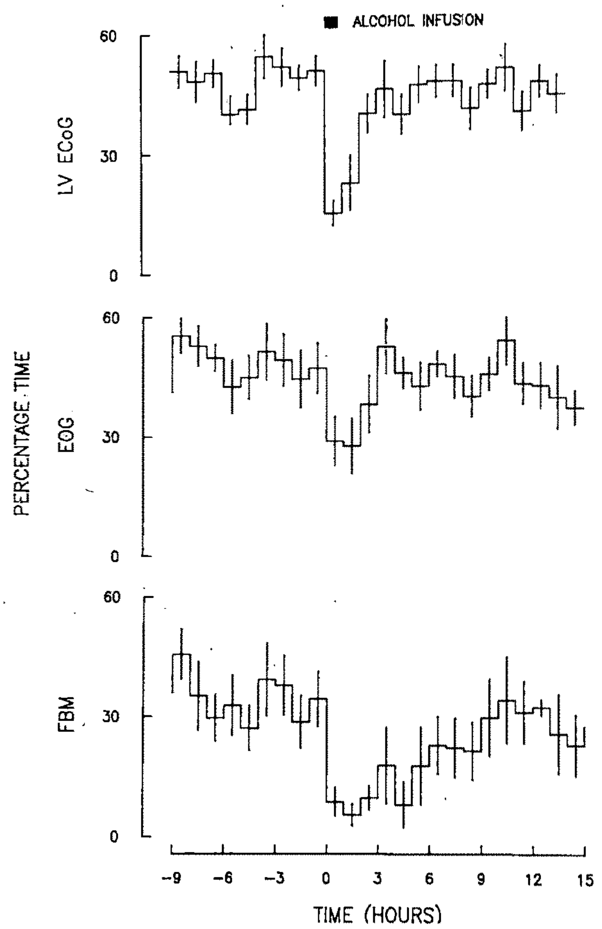

Fig. 6. The incidence of low-voltage electrocortical activity *(LV ECoG)*, electroocular activity *(EOG)*, and fetal breathing movements *(FBM)* was plotted each hour (±SEM) before or after alcohol infusion.

to account for the marked decrease in the incidence of fetal breathing following ethanol administration.

In human fetuses McLeod et al.[5] reported that oral administration of ethanol (0.25 gm/kg) to pregnant women near term virtually abolished fetal breathing activity for at least 3.5 hours. In the current study on third-trimester pregnant ewes, maternal intravenous infusion of 1 gm/kg of ethanol over 1 hour was used to reproduce similar effects on fetal breathing activity. The rate of ethanol elimination from fetal arterial blood was 0.14 mg/ml/hr, which was similar to the rate of elimination from maternal arterial blood in sheep (0.12 mg/ml/hr)[18] and maternal venous blood in humans (0.15 mg/ml/hr).[13] It appears that the chronic fetal lamb preparation is a useful model for studying the effects of ethanol on fetal life and that the pharmacokinetics of ethanol are similar in the human, based on maternal ethanol elimination rates.

During human pregnancy it is impossible to study the effects of ethanol on fetal brain function or metabolism. The current results suggest that when ethanol is administered to pregnant ewes, similar suppressive effects on fetal breathing activity are observed to those reported in women.[4, 5] Furthermore, in this study, remarkable changes occurred in fetal electrocortical activity and eye movements and a different and unusual type of intermediate-voltage electrocortical activity occurred immediately following ethanol administration. Finally, preliminary analysis of other data from this experiment suggests that significant decreases occurred in fetal brain blood flow and oxidative metabolism following maternal ethanol administration.[10] It would seem prudent for pregnant women to refrain from consumption of ethanol during pregnancy.

We wish to thank Drs. J. Challis, P. Harding, and D. Olson for their interest in this work and Mrs. T. Clarke for her excellent technical assistance.

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Discussion

DR. MORTIMER G. ROSEN, Cleveland, Ohio. The authors have made an orderly progression from observations on humans to observations on animals in order to study the physiology and possible pathologic physiology relating to the use of ethyl alcohol, which is a drug of major societal interest. This is an observational or descriptive study, since there were no stated hypotheses.

In previous studies of pregnant women the authors documented that after one alcoholic drink, fetal breathing movements were suppressed for 30 minutes and the suppression lasted for about 3 hours. Fetal heart rate and fetal body movements were not altered.

The present study of fetal lambs offers an excellent opportunity to describe the many behavioral and metabolic changes that may take place in the presence of an alcohol infusion. The amount of alcohol given in this study and measured intravenously in the fetus during the first hour exceeded the lower limits for light adult drinking in the human. For example, the threshold for measurable impairment for human subjects who are light drinkers ranges from 0.5 to 0.9 mg/ml in blood, and the probability for causing an automobile accident doubles at the 0.6 mg/ml level and increases over 25 times at the 1.5 mg/ml level, which level was exceeded in this fetal sheep study.[1]

The findings documented changes in almost all systems, except the acid/base status and body movements. The fetal breathing movements appear to have remained suppressed long after electrocortical and heart rate changes returned to pretesting measurements.

That there was a major change in electrocortical activity is not a surprise. Many drugs that are fat soluble, including alcohol, narcotics, tranquilizers, and barbiturates, alter states of consciousness and are easily seen in the electrocorticogram. Drugs do not have specific electrical signatures but instead alter electrical potentials in similar patterns. The observed changes probably relate to alcohol acting on the lipid membranes. The authors state "therefore the suppression of fetal breathing movements by maternal ethanol infusion could not be explained by changes in electrocortical activity." Since the findings are a summation of electrical activity in the cortex (as well as deeper structures), this test cannot be used to define control. While the cortex modulates and even controls activities such as heart rate and breathing, one cannot equate change with control. The best example of this is the disparity noted when a person with a flat electroencephalogram may be legally defined as dead but can still maintain respiratory and cardiovascular activity.

There also is a danger in proceeding from an observed change in variables to the proscription of alcohol use. One may talk about limitation in the use of alcohol, which is certainly appropriate for the fetus as well as the rest of us, but the fetal internal environment responds to many stimuli.

For example, in the authors' previous studies we note that club soda also altered the electrocorticogram. Should club soda be restricted? Similarly glucose alters fetal activity. Is that good or bad? The fetal environment is extremely sensitive. In this study during the three hours of the control period before the alcohol infusion began, the fetal heart rate increased, perhaps reflecting the ewe's response to the activity in the laboratory and the fetal response to the ewe's response. Was that good or bad, or was it part of normal early fetal brain development?

After birth each fetus is seen as a unique person, different from any other fetus in the neonatal nursery or in that patient's family. This is because the internal milieu has been different for that fetus. Almost every thing the mother does, including physical activity, metabolic alterations, sleep and emotional behavior, and even sounds of the external world, will alter the electrocorticogram and perhaps alter fetal breathing movements. Years ago during fetal sheep studies we placed auditory transducers at the fetal sheep ear and heard the sounds of the fetal environment including noise transmitted when we percussed the maternal abdomen.

I do have several specific questions that I would pose for Dr. Patrick and co-workers. (1) Do you have any opinion at this time as to how useful fetal breathing movements may be in detecting fetal distress, since the response times as noted in this study were quite different from fetal movement, heart rate, and corticogram changes? (2) Is it possible to analyze the electrocorticogram more specifically than in terms of high, low, and intermediate voltage? If you run at faster recording paper speeds would you be able to define fetal sleep behavioral patterns similar to patterns present in the human fetus and neonate? (3) Why do you feel that when one signal is suppressed longer than another (i.e., fetal breathing movement as compared to electrocorticogram), it is or is not controlled by the other signal? (4) Do you feel one glass of an alcoholic drink or alcohol in any amount is harmful and should be interdicted during pregnancy?

Once again, I congratulate Dr. Patrick and colleagues for these well-designed studies, which were set forth in a concise and clear manner.

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DR. SAMUEL SOLOMON, Montreal, Quebec, Canada. Does the pregnant sheep have alcohol dehydrogenase in the maternal or fetal circulation? Can this enzyme be induced in the mother or fetus? If there is little or no dehydrogenase the alcohol can alter membrane structure and perhaps explain some of the results you obtained.

Dr. Patrick (Closing). Dr. Rosen's first concern was regarding the amount of alcohol given during this experiment. We spent an entire sheep season working out this dose. There is a major species difference in the effect of alcohol on fetal breathing activity between the human and the sheep. The dose used in this study reproduces the effects on fetal breathing movements that we reported in the human when we administered 0.25 gm/kg of body weight to human pregnant volunteers.[5]* There may be a difference in alcohol dehydrogenase activity, or it may just be a pure species difference. I do not know. We do know that the elimination rate of ethanol in maternal blood from our studies in the human and in the sheep are very similar.[5, 18]

In regard to the fetal brain waves, Leon Mann first noted this electrocortical change when he performed acute studies on the effects of ethanol on the fetus many years ago.[15] We repeated, initially, the doses that were used by Mukherjee and Hodgen[17] in Rhesus monkeys. These more rapid chart recordings of electrocortical activity were obtained after administration of 3 gm of ethanol per kilogram of maternal body weight, not over 2 minutes as Mukherjee and Hodgen reported, but over 1 hour. There is a progression of electrocortical activity in the fetus to complete absence of voltages. It is the only experimental condition I have yet seen which produces an isoelectric electrocorticogram in an animal that subsequently recovered.

I do think that our human studies suggest that club soda doesn't alter electrocortical activity or fetal breathing movements.[5] It was unfortunate that in the first study,[5] the time base on the figures suggested a change in fetal breathing movements, but it really reflected a gradual change after breakfast in those women who were not fasting.

The fetal heart rate did not change in the control animal in the present study or in similar studies performed for other reasons in animals that were not infused with ethanol, so I do not think it was the conditions of the experiment that contributed to the present observations.

I cannot give you an opinion on how useful fetal breathing movements are in the prediction of fetal distress in the human. There are increasing numbers of variables known to affect human fetal breathing activity that do not influence fetal body movements or accelerations in the fetal heart rate near term. Certainly an important one is the impending onset of labor, and as far as we know, there is no way of knowing how close labor is in humans. Our human studies[5, 6] suggested that if a woman had a cocktail for lunch and was tested 2 hours later, by which time her breath would not even smell of alcohol, her fetus would still not be making any breathing movements. However, the next morning her fetus would be perfectly well from the point of view of fetal breathing activity. Fetal breathing may be a sensitive indicator of hypoxemia over long-term observations, but from the practical human point of view we have to make observations over such a short interval, perhaps an hour at most, that I do not think at the present time the measurement has a major role to play in detection of fetal distress in humans.

I agree it is possible to analyze the fetal electrocorticogram in other ways. A combination of spectral techniques and of voltages that are demonstrated here could be used, but for this initial report we chose to show voltage levels only.

Is one glass of alcohol harmful to pregnant women? I do not have an answer except that there is a remarkable effect in the human for a very long period of time on one biologic marker of health, namely fetal breathing. However, fetal breathing movements in our human studies had returned to a normal incidence by the following day and all of the study fetuses were normal at birth.[5, 6] These studies have a rather sobering effect in our recommendations to patients. It remains to be seen whether extensions of this study will suggest that repeated alcohol infusions will induce protective mechanisms such as alcohol dehydrogenase activity.

I will now discuss Dr. Solomon's questions. Alcohol is inactivated by a dehydrogenase enzyme, and this is a key factor we are looking at in the current year: Can we induce fetal alcohol dehydrogenase function? There is no arteriovenous difference in alcohol concentration across the fetal brain in this model. Not surprising perhaps, because it is known that in the human there is virtually no alcohol dehydrogenase activity in the brain. We had no evidence in this study that there is any alcohol dehydrogenase activity in the fetus. We demonstrated zero-order alcohol kinetics in amniotic fluid and fetal and maternal blood. Blood ethanol concentrations were similar in fetal and maternal blood, and we measured an elimination rate in the amniotic cavity similar to that of the mother and the fetus.

We did measure acetaldehyde.[18] We tended to detect acetaldehyde levels primarily during the time of the electrocortical changes, that is, the first 3 to 4 hours, and we are pursuing this idea now. I agree that acetaldehyde is toxic, which may explain some of the findings in this study.

Effects of ethanol on cell membranes may relate to controls of fetal breathing. There are central controls that are probably located in the level of the fetal lamb brain below the upper pons. There are, in air-breathing people and of course in fetuses, peripheral controls such as carotid chemoreceptors and aortic and pulmonary stretch receptors. In the sheep there is evidence that the carotid chemoreceptor in fetal life is not functional. There is evidence that the central chemoreceptors are functional. Fetal breathing is also mediated by electrocortical activity, and Dawes et al.[16] have demonstrated that if you section the fetal brain at the level of the upper pons, thereby removing the effect of electrocortical activity from central control, fetuses go on breathing episodically but not in relationship to electrocortical activity. Dawes has recently shown that maternal ethanol administration in roughly the same doses as the current study given to brain sectioned preparations also caused suppression of fetal breathing activity. Alcohol and prostaglandin E_2 are the only two substances we know at present that will suppress fetal breathing activity in lambs with caudal brain stem section. This is one of the reasons that we suggested that the effect of ethanol on fetal breathing activity is central. The current data, as well as Dawes' brain stem section experiments, suggest that ethanol has a central effect and not a secondary effect to an influence on fetal electrocortical activity.

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*The references in this discussion are given at the end of the original paper.

Effects of ethanol on cell membranes may be important. Prostaglandin E_2 is a potent suppressor of fetal breathing. It is important to remember that one of the key problems for people in tissue culture work when they add ethanol to preparations of cell membranes is the large production of prostaglandins.

Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol

Uwe Goebelsmann, M.D.,† C. Ann Mashchak, M.D., and Daniel R. Mishell, Jr., M.D.
Los Angeles, California

The pronounced hepatic impact of oral ethinyl estradiol has been attributed by some to its so-called first-pass effect through the liver as only some 40% of ingested ethinyl estradiol reaches the systemic circulation. Others believe that ethinyl estradiol exerts its hepatic effects because of its chemical composition, specifically its 17α-ethinyl group. In an attempt to resolve this controversy, a study was undertaken to determine whether vaginal administration of ethinyl estradiol can selectively reduce the hepatic effects of oral ethinyl estradiol. To compare the effects of oral and vaginal ethinyl estradiol, a group of postmenopausal subjects received either 5 μg of oral and 20 μg of vaginal ethinyl estradiol or 10 μg of oral and 50 μg of vaginal ethinyl estradiol in either sequence, respectively. Oral ethinyl estradiol was four to five times more potent than vaginal ethinyl estradiol. The potency ratios of the oral-vaginal ethinyl estradiol doses required to suppress follicle-stimulating hormone and luteinizing hormone were 4.4 and 3.2 and those to raise sex hormone–binding globulin binding capacity, corticosteroid-binding globulin binding capacity, and high-density lipoprotein cholesterol as well as lower low-density lipoprotein cholesterol were 3.5, 5.0, 4.2, and 4.2, respectively. These essentially equal oral-vaginal route potency ratios for both central nervous system and hepatic effects indicate that vaginal administration of ethinyl estradiol does not selectively reduce its hepatic impact in relation to its central nervous system effects. The pronounced hepatic effects of ethinyl estradiol are therefore attributed to its chemical composition. (AM J OBSTET GYNECOL 1985;151:868-77.)

Key words: Ethinyl estradiol, sex hormone–binding globulin binding capacity

Ethinyl estradiol, as shown in a recent report from our laboratory, exerts a much more pronounced hepatic effect in relation to its gonadotropin suppressive activity than is observed following the administration of piperazine estrone sulfate, micronized estradiol, or conjugated equine estrogens.[1] This pronounced effect of ethinyl estradiol upon hepatic protein synthesis has been attributed to its approximately 60% first-pass effect through the liver following oral administration.[2] Other investigators, however, presented evidence that vaginal administration of ethinyl estradiol failed to reduce its effect upon hepatic corticosteroid-binding globulin (CBG) synthesis despite the fact that the initial enterohepatic passage was avoided.[3] However, the latter investigators did not directly compare the effects of orally and vaginally administered ethinyl estradiol upon CBG binding capacity (CBG-BC) with those upon circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. Furthermore, other well-established parameters of hepatic ethinyl estradiol effects, such as serum sex hormone–binding globulin binding capacity (SHBG-BC)[4] or high-density lipoprotein and low-density lipoprotein cholesterol, have not been examined after vaginal ethinyl estradiol administration or compared with those observed after ethinyl estradiol ingestion. In addition, the bioavailability of vaginally administered ethinyl estradiol remained to be investigated while the pharmacokinetics of vaginally administered micronized 17β-estradiol have been studied[5] and compared with those of orally administered 17β-estradiol.[6]

Thus the objectives of this study were (1) to assess the bioavailability of vaginally administered ethinyl estradiol and compare it with that of ingested ethinyl estradiol[7] and (2) to determine the effects of two different doses of oral and vaginal ethinyl estradiol of comparable bioavailability upon serum FSH and LH concentrations as well as CBG-BC, SHBG-BC, high-density lipoprotein and low-density lipoprotein cholesterol, and triglycerides in an effort to ascertain whether parenteral ethinyl estradiol administration selectively reduces its hepatic effects in relation to its gonadotropin suppressive activity in postmenopausal women.

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Material and methods

Preparation of ethinyl estradiol capsules and suppositories. Ethinyl estradiol capsules were prepared as follows in an effort to assure an accurate dosage of oral ethinyl estradiol. Tablets of 50 μg Feminone (The Upjohn Co.) were ground and mixed with lactose. Aliquots equivalent to 5 and 10 μg of ethinyl estradiol were weighed and placed into gelatin capsules.

Ethinyl estradiol suppositories were prepared from polyethylene glycol and authentic, twice recrystallized ethinyl estradiol. Polyethylene glycol 400 and 6000, both United States Pharmacopeia grade, were mixed in a weight ratio of 3:2 to serve as the base for suppositories weighing 2.4 gm each. Ethinyl estradiol, 25 mg, was dissolved in 1 ml of United States Pharmacopeia absolute ethanol and diluted with polyethylene glycol 400 to a total of 25 ml in a volumetric flask. To prepare 100 suppositories, each containing 20, 50, or 120 μg of ethinyl estradiol, 2, 5, and 12 ml of the ethinyl estradiol solution were combined with aliquots of the polyethylene glycol base and heated to a liquid state, for a total weight of 240 gm for each batch. After careful mixing with a magnetic stirrer, the heated solutions were poured into the suppository molds which had been tested to yield exactly 2.4 gm suppositories. The filled molds were allowed to cool. The ethinyl estradiol suppositories were stored in glass jars at 4° C and used within 12 months after being manufactured.

Subjects. Five healthy women voluntarily participated in the initial phase of this study, during which the bioavailability of vaginal ethinyl estradiol was determined. These volunteers were 26 to 31 years old, were 157 to 166 cm tall, weighed 47.7 to 74.2 kg, had regular menstrual cycles, and used nonhormonal means of contraception at the time of the study and within the preceding 3 months. The study was performed in each subject during the early follicular phase, as soon as menstruation had ceased. The subjects were in a supine position during the first 12½ hours of the study. An indwelling needle was inserted into an arm vein between 7:30 and 8:00 AM and blood was withdrawn at −30, −15, and 0 minutes to determine the average serum ethinyl estradiol "blank." A suppository containing 120 μg of ethinyl estradiol was inserted deep into the vagina at 0 minutes and venous blood was obtained 30 minutes and 1, 1½, 2, 3, 6, 12, and 24 hours thereafter. The blood was allowed to clot for 1 hour at room temperature and then centrifuged. The serum was stored at −20° C until analyzed for ethinyl estradiol.

Nine healthy postmenopausal women volunteered for the main phase of this investigation, during which the effects of vaginally administered ethinyl estradiol upon serum gonadotropins, SHBG-BC, and CBG-BC as well as lipids were compared with those of orally administered ethinyl estradiol. These volunteers were 48 to 67 years old, were 148 to 164 cm tall, and weighed 47.7 to 88.6 kg. None of the nine volunteers had received any estrogen within 6 months prior to the beginning of this study. They were randomly assigned to receive either a larger or a smaller dose of oral and vaginal ethinyl estradiol, each given over a course of 25 consecutive days. There was a 6-week estrogen-free interval between each subject's course of oral and vaginal ethinyl estradiol, the sequence of which was randomized as well. Oral ethinyl estradiol was administered as either 5 or 10 μg capsules while vaginal ethinyl estradiol was given as either 20 or 50 μg suppositories. Ingestion or vaginal administration took place at the time the volunteers retired to bed for 25 consecutive nights. As a result of randomization, two of the nine subjects received 10 μg of ethinyl estradiol orally followed by 50 μg of vaginal ethinyl estradiol, one volunteer received 50 μg of vaginal ethinyl estradiol followed by 10 μg of oral ethinyl estradiol, two volunteers were given 5 μg of oral ethinyl estradiol followed by 20 μg of vaginal ethinyl estradiol, and four subjects were administered 20 μg of ethinyl estradiol vaginally followed by 5 μg of oral ethinyl estradiol. Antecubital venous blood was drawn at 8:00 AM after an overnight fast on 2 subsequent days immediately prior to the first ethinyl estradiol dose and on days 16 and 17 as well as days 23 and 24 following the first ethinyl estradiol administration. All blood samples except for total, high-density lipoprotein, and low-density lipoprotein cholesterol and triglyceride assays were allowed to clot for 1 hour, after which the serum was separated by centrifugation and stored at −20° C until analyzed. Blood for the latter assays was collected into tubes containing ethylenediaminetetraacetic acid and the plasma was separated immediately and transferred without delay for analysis.

Assays. Serum ethinyl estradiol was measured by radioimmunoassay as previously described by us.[8] In brief 6,7-tritium–labeled ethinyl estradiol with a specific activity of 55 Ci/mmol was purchased from New England Nuclear, Boston, Massachusetts, and checked for radiochemical purity by paper partition chromatography as previously described.[8] A 0.1 ml aliquot of tritium-labeled ethinyl estradiol (1000 dpm) in phosphate gelatin buffer consisting of 0.15 mol/L of sodium chloride, 0.1 mol/L of sodium phosphate at pH = 7.0, 0.1% (w:v) Knox unflavored gelatin, and 0.1% (w:v) sodium azide was added to 1 ml serum aliquots. After a 30-minute incubation at 37° C, the serum was extracted twice with 5 ml of diethyl ether. The combined ether extracts were evaporated and the residue was subjected to Celite partition chromatography. The ethinyl estradiol fraction was evaporated and the residue redissolved in 0.7 ml of phosphate gelatin buffer. Duplicate

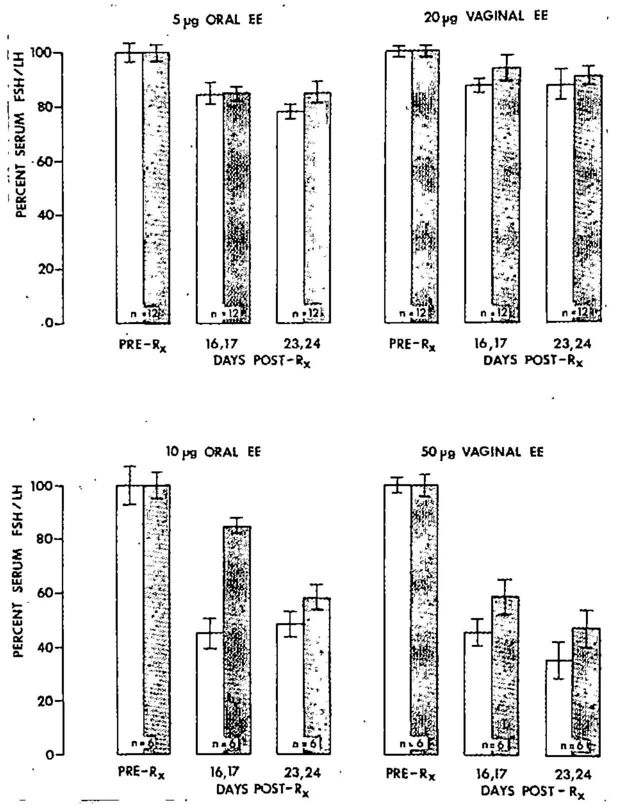

Fig. 1. Mean (±SE) serum FSH *(open columns)* and LH *(hatched columns)* concentrations in percentage of pretreatment *(PRE-R$_x$)* levels prior to and 16 and 17 as well as 23 and 24 days following daily oral or vaginal administration *(POST-R$_x$)* of 5 and 20 or 10 and 50 µg of ethinyl estradiol *(EE)*, respectively, in postmenopausal women.

0.2 ml aliquots were submitted to radioimmunoassay and a third 0.2 ml aliquot was subjected to liquid scintillation counting to assess procedural losses. The radioimmunoassay was performed with the above-mentioned 6,7-tritium–labeled ethinyl estradiol, recrystallized authentic ethinyl estradiol (Steraloids, Inc., Wilton, New Hampshire) used as standard, and an anti–ethinyl estradiol-7-(3-thiopropionic acid)-bovine serum albumin serum, which was provided by Dr. C. E. Cook, Research Triangle Institute, Research Triangle Park, North Carolina. Dextran-coated charcoal was used to separate bound and unbound tritium-labeled ethinyl estradiol. An eight-point standard curve ranging from 3.9 to 500 pg was run in duplicate in each assay and logit transformation was used to construct standard curves and calculate unknowns on a programmable Hewlett-Packard Model 97 calculator. The sensitivity of this radioimmunoassay is 20 pg of ethinyl estradiol per milliliter of serum. During the course of this study, monitoring of precision and accuracy by measurement of aliquots of three plasma pools containing 50, 100, and 200 pg of ethinyl estradiol per milliliter revealed interassay coefficients of variation of 9.6%, 6.1%, and 8.0%, respectively, while, on the average, 108.8% ± 5.9% of the ethinyl estradiol present in the above pools was "recovered" after correction for procedural loss by the use of the above internal standard. Buffer blanks were invariably indistinguishable from zero while serum samples from subjects in the early midfollicular phase who had not received ethinyl estradiol or mestranol averaged 22 ± 6 pg/ml. This "serum" blank is due to the 28% cross-reaction of 17β-estradiol with the antiserum in the presence of small amounts of ethinyl estradiol as previously described.[8] Therefore, the serum blank was determined in three different samples from each volunteer before they received ethinyl estradiol. These readings were averaged and subtracted from all ethinyl estradiol levels measured during the course of this study.

Table I. Mean (±SE) serum ethinyl estradiol concentrations in five women*

Time after suppository insertion	*Serum ethinyl estradiol concentrations (pg/ml)*
30 min	22.8 ± 6.4
60 min	43.9 ± 11.1
90 min	56.7 ± 12.1
3 hr	132 ± 33.2
6 hr	102 ± 8.1
12 hr	51.6 ± 11.2
24 hr	25.0 ± 9.2

*Measurements during early follicular phase of apparently ovulatory cycle following vaginal administration of polyethylene glycol suppository containing 120 µg of ethinyl estradiol.

Serum LH and FSH levels were determined by double-antibody radioimmunoassays analogous to procedures reported by Midgley.[9]

SHBG-BC and CBG-BC were determined as previously reported from this laboratory.[10, 11] SHBG-BC was calculated as the mass of steroid (tritium-labeled dihydrotestosterone) capable of being specifically bound to SHBG and expressed in nanomoles per liter while CBG-BC was reported as microgram equivalents of tritium-labeled cortisol bound per 100 ml of serum. Total, high-density lipoprotein, and low-density lipoprotein cholesterol as well as triglycerides was determined in plasma containing ethylenediaminetetraacetic acid by Bio-Science Laboratories, Van Nuys, California.[12, 13] Two-tailed Student's *t* test was used for statistical analysis.

Results

Bioavailability of vaginally administered ethinyl estradiol. The serum ethinyl estradiol concentrations observed following the administration of a vaginal suppository containing 120 µg of ethinyl estradiol in 2.4 gm of polyethylene glycol to five normally menstruat-

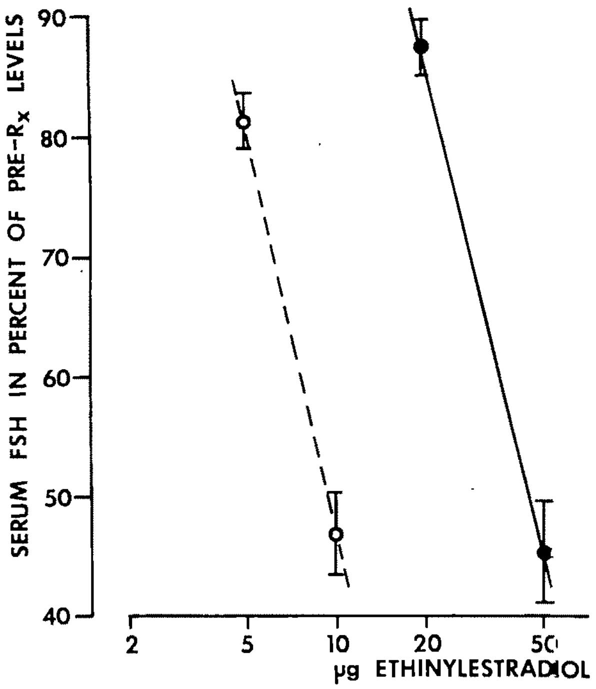

Fig. 2. Mean (±SE) serum FSH concentrations, expressed as percentage of pretreatment levels *(PRE-R_x)*, observed 16, 17, 23, and 24 days following daily oral *(circles, dashed line)* and vaginal *(dots, solid line)* administration of 5 or 10 and 20 or 50 µg of ethinyl estradiol, respectively, in postmenopausal women.

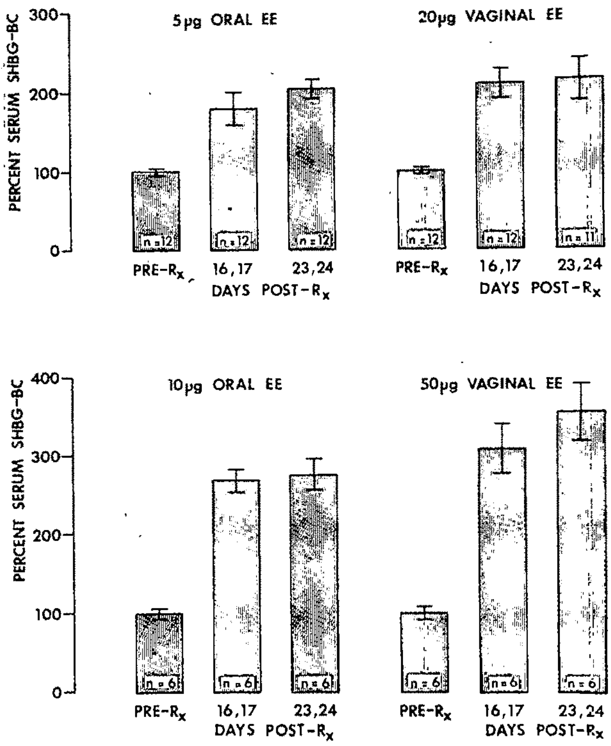

Fig. 3. Mean (±SE) serum SHBG-BC in percentage of pretreatment *(PRE-R_x)* levels prior to and 16 and 17 as well as 23 and 24 days following daily oral or vaginal administration *(POST-R_x)* of 5 and 20 or 10 and 50 µg of ethinyl estradiol *(EE)*, respectively, in postmenopausal women.

ing women in the early follicular phase are listed in Table I.

Comparison of oral and vaginal ethinyl estradiol effects. The gonadotropin suppressive effects of 5 µg of oral versus 20 µg of vaginal ethinyl estradiol and of 10 µg of oral versus 50 µg of vaginal ethinyl estradiol upon six and three postmenopausal women, respectively, are depicted in Fig. 1. The mean serum FSH levels observed on the sixteenth and seventeenth as well as twenty-third and twenty-fourth days of the 25-day course of either of two doses of oral or vaginal ethinyl estradiol were significantly lower ($p < 0.01$ at the two lower and $p < 0.005$ at the two higher doses of oral and vaginal ethinyl estradiol) than pretreatment serum FSH concentrations, which averaged 70.8 ± 4.3 mIU/ml (±SE). Log dose versus response curves for both routes of administration were virtually parallel (Fig 2). The regression lines calculated for the logarithms of the two oral and the two vaginal ethinyl estradiol doses (x) and for the corresponding percentage of decrease in serum FSH (y) were $y = -114.75x + 61.59$, $r = 0.8167$, $n = 35$, and $y = -118.35x + 141.38$, $r = 0.8811$, $n = 36$, respectively. As calculated from these regression lines, a 50% decrease in serum FSH is achieved by 9.4 µg of oral ethinyl estradiol or by 41.4 µg of ethinyl estradiol administered as a polyethylene glycol suppository resulting in a potency ratio of 4.4:1 for oral versus vaginal ethinyl estradiol. All mean serum LH concentrations measured on the sixteenth and seventeenth as well as twenty-third and twenty-fourth days of either 5 or 10 µg of ethinyl estradiol by mouth or 20 or 50 µg of ethinyl estradiol by vaginal suppository with the exception of that obtained on days 16 and 17 of the 25-day course of 20 µg of vaginal ethinyl estradiol daily were significantly reduced ($p < 0.025$) from pretreatment levels, which averaged 57.4 ± 5.9 mIU/ml. The log dose versus response (percentage of decrease in serum LH) curve for orally administered ethinyl estradiol did not parallel that for vaginal ethinyl estradiol precluding calculation of an exact potency ratio. However, based on a comparison of the percentages of LH suppression achieved by the two oral and the two vaginal ethinyl estradiol dose regimens, the approximate potency ratio of orally administered–vaginally administered ethinyl estradiol is 3.2.

SHBG-BC increased significantly ($p < 0.005$) in response to both the 5 and 10 µg oral doses as well as the 20 and 50 µg vaginal doses from pretreatment values, which averaged 57.1 ± 4.0 nmol/L (Fig. 3). The regression lines calculated for the logarithms of the two

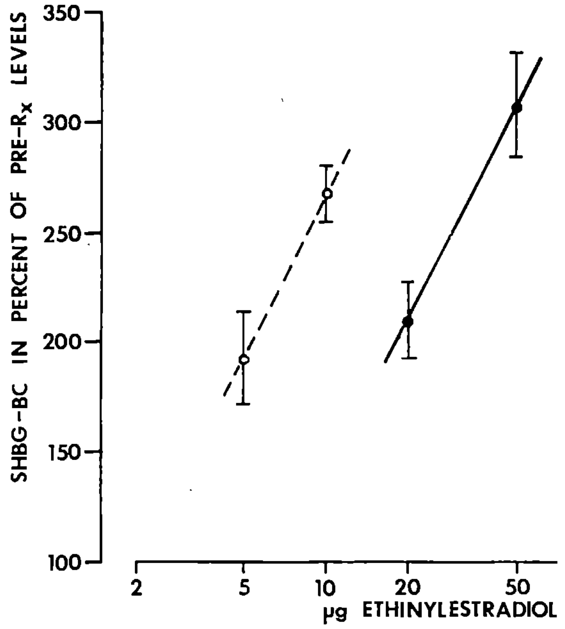

Fig. 4. Mean (±SE) serum SHBG-BC, expressed as percentage of pretreatment *(PRE-R_x)* levels, observed 16 and 17 days after daily oral *(circles, dashed line)* and vaginal *(dots, solid line)* administration of 5 or 10 and 20 or 50 μg of ethinyl estradiol, respectively, in postmenopausal women.

oral and the two vaginal ethinyl estradiol doses (x) and for the corresponding SHBG-BC values (y) observed on days 16 and 17 of the 25-day course of ethinyl estradiol administration and expressed as the percentage of rise over pretreatment levels were $y = 250.6x - 82.5$, $r = 0.6141$, $n = 18$, and $y = 246.3x - 211.3$, $r = 0.5927$, $n = 18$ (Fig. 4). From these regression lines it was calculated that 5.3 and 13.4 μg of oral ethinyl estradiol and 18.4 and 46.8 μg of vaginal ethinyl estradiol, when administered daily for 17 days, would raise SHBG-BC by 100% and 200%, respectively. Based on the virtual parallelism of the dose-response curves, a potency ratio of 3.5 was calculated for oral ethinyl estradiol capsules versus vaginal ethinyl estradiol suppositories. The dose-response curves for SHBG-BC values obtained on days 16 and 17 as well as on days 23 and 24 combined were not parallel because of the higher results observed on days 23 and 24 in subjects receiving the 50 μg dose of vaginal ethinyl estradiol. However, the latter results did not differ significantly from the SHBG-BC levels obtained on days 16 and 17. Based on the combined data, the oral-vaginal ethinyl estradiol potency ratio would be 3.6 for doses achieving a 100% increase and 3.0 for doses raising SHBG-BC by 200%.

CBG-BC rose significantly ($p < 0.005$) in response to 5 and 10 μg of oral as well as 20 and 50 μg of vaginal ethinyl estradiol from a mean (±SE) pretreatment level of 17.3 ± 0.8 μg/100 ml (Fig. 5). The dose-response curves describing the rise of CBG-BC following oral and vaginal ethinyl estradiol, respectively, were not parallel. While the 59% ± 3.6% (n = 12) rise of CBG-BC in response to 50 μg of vaginal ethinyl estradiol did not differ from the 55% ± 3.4% (n = 12) CBG-BC increase after 10 μg of oral ethinyl estradiol, the 50% ± 8.5% rise after 5 μg of oral ethinyl estradiol was significantly higher than the mere 22.4% ± 3.8% increase following the 20 μg of ethinyl estradiol vaginal suppository. Based on the virtually equal CBG-BC response to 10 μg of oral and 50 μg of vaginal ethinyl estradiol, the oral-vaginal ethinyl estradiol potency ratio is estimated as being approximately 5.

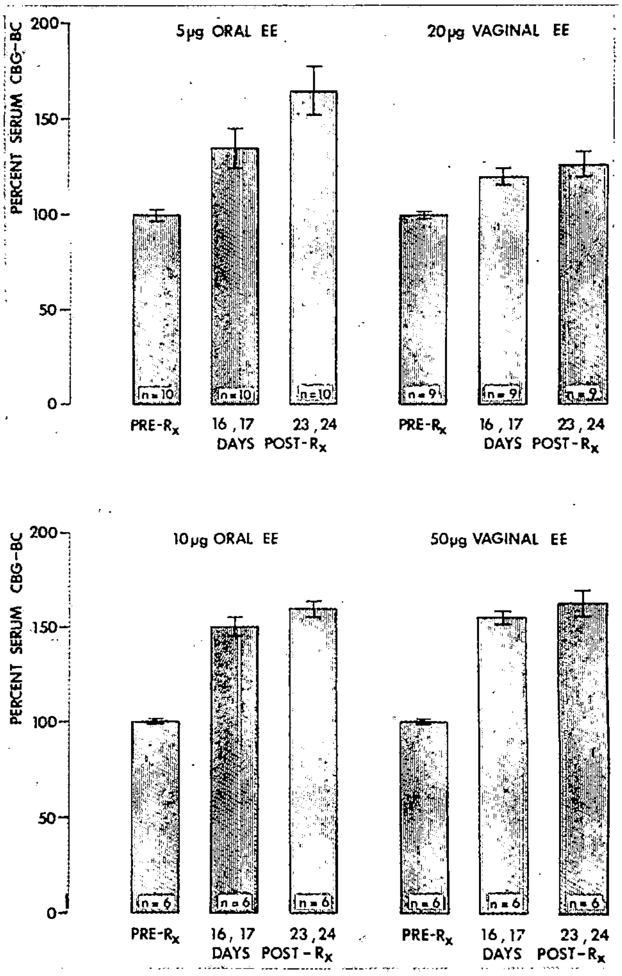

Fig. 5. Mean (±SE) serum CBG-BC in percentage of pretreatment *(PRE-R_x)* levels prior to and 16 and 17 as well as 23 and 24 days following daily oral and vaginal administration *(POST-R_x)* of 5 and 20 or 10 and 50 μg of ethinyl estradiol *(EE)*, respectively, in postmenopausal women.

Total cholesterol and triglyceride concentrations averaging 248 ± 17 and 136 ± 18 mg/100 ml, respectively, were not altered significantly during the 25-day course of the two doses of oral or vaginal ethinyl estradiol (Fig. 6). The high-density lipoprotein cholesterol levels, however, rose significantly from a pretreatment mean of 48 ± 2.5 mg/100 ml following 10 µg ($p < 0.001$) and 5 µg ($p < 0.02$) of oral and 50 µg ($p < 0.01$) of vaginal ethinyl estradiol yet were not significantly elevated following 20 µg of vaginal ethinyl estradiol (Fig. 6). The dose-response curves calculated for the rise in high-density lipoprotein cholesterol following oral and vaginal ethinyl estradiol, respectively, were not parallel. It was calculated that a 10% rise in high-density lipoprotein cholesterol was obtained with about 5 and 18.5 µg of oral or vaginal ethinyl estradiol and that a 25% increase would require about 9.6 and 44 µg of oral and vaginal ethinyl estradiol, respectively. These data would allow approximation of the oral-vaginal ethinyl estradiol potency ratio as 4.2. The low-density lipoprotein cholesterol levels, which averaged 173 ± 17 mg/100 ml prior to ethinyl estradiol administration, decreased significantly ($p < 0.05$) only after 10 µg of oral or 50 µg of vaginal ethinyl estradiol (Fig. 6). Dose-response curves for oral and vaginal ethinyl estradiol were not parallel, thus precluding the assessment of the oral-vaginal ethinyl estradiol efficacy ratio. However, it can be approximated that about 10 µg of oral and 42 µg of vaginal ethinyl estradiol caused a 20% decrease in low-density lipoprotein cholesterol, allowing estimation of the oral-vaginal potency ratio as approximately 4.2.

Comment

The serum ethinyl estradiol levels achieved following vaginal administration of 120 µg of ethinyl estradiol homogeneously incorporated in a 2.4 gm polyethylene glycol suppository were much lower than those found by us in a previous study where the same dose of ethinyl estradiol was ingested by 24 women[7] who matched the present five study subjects with regard to age, weight, and height (Fig. 7). The latter study was performed in this laboratory and serum ethinyl estradiol concentrations were measured by the same technologist using the same procedure, reagents, standard, and controls. The area delineated by the serum ethinyl estradiol concentrations *(ordinate)* and the time after administration *(abscissa)*, commonly regarded as a measure of bioavailability, after vaginal ethinyl estradiol administration amounted to merely 36% of that found after ethinyl estradiol ingestion. As Fig. 7 indicates, the mean serum ethinyl estradiol concentrations found after vaginal ethinyl estradiol administration were significantly lower than those achieved by ingesting the same amount of ethinyl estradiol ($p < 0.001$ for all except the 24-hour values with $p < 0.02$). Our finding of a significant decrease in bioavailability following vaginal ethinyl estradiol administration is in apparent disagreement with that of Schwartz et al.[3] The latter authors reported that serum ethinyl estradiol levels measured 8 to 10 hours following vaginal insertion of 20 to 30 µg ethinyl estradiol tablets were in the same range (23 to 131 pg/ml) as those published by others[14] following ingestion of 50 µg ethinyl estradiol tablets (25 to 131 pg/ml). A likely explanation for this apparent discrepancy is that vaginal ethinyl estradiol absorption is less efficient if the ethinyl estradiol is contained in a polyethylene glycol suppository than when it is inserted as a tablet. Not only were the peak serum ethinyl estradiol levels, on the average, four times lower but the peak serum ethinyl estradiol concentrations were reached later after vaginal administration, compared to oral administration, of the same 120 µg dose of ethinyl estradiol (at 3 instead of 1½ hours). However, the rate of decline of serum ethinyl estradiol levels after the peak had been reached was more protracted when ethinyl estradiol was given by the vaginal instead of the oral route. This slower fall of serum ethinyl estradiol concentrations

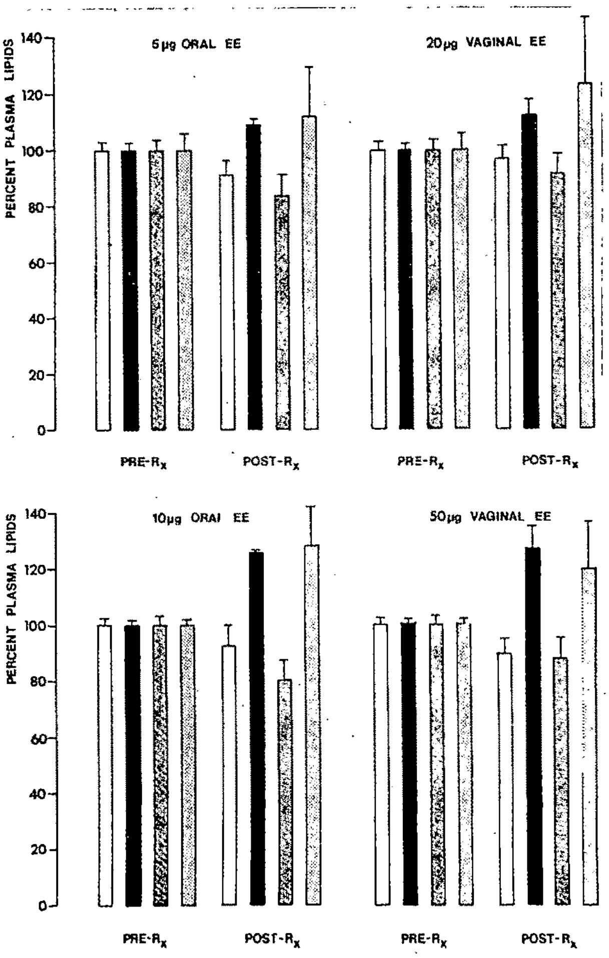

Fig. 6. Mean (±SE) plasma total *(open columns)*, high-density lipoprotein *(solid columns)* and low-density lipoprotein cholesterol *(hatched columns)* as well as triglycerides *(dotted columns)* prior to *(PRE-R_x)* and 24 days after daily oral and vaginal administration *(POST-R_x)* of 5 or 10 and 20 or 50 µg of ethinyl estradiol *(EE)*, respectively, in postmenopausal women.

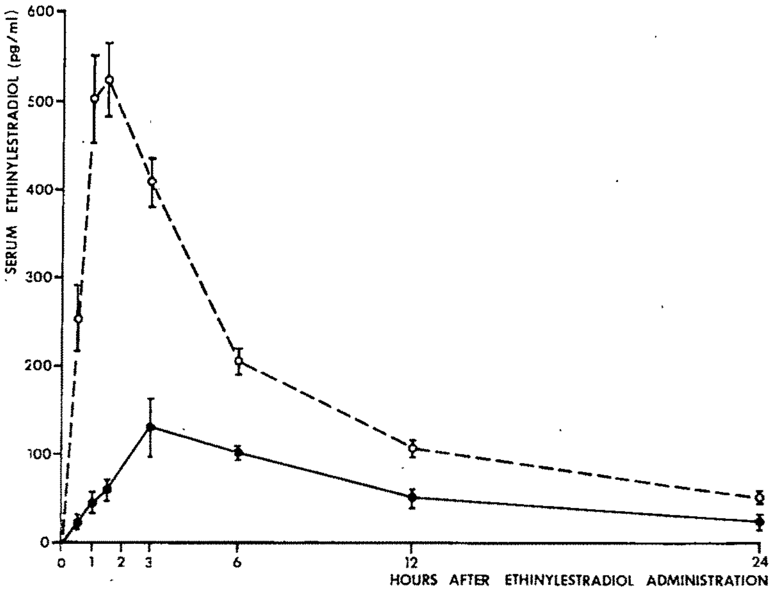

Fig. 7. Mean (±SE) serum ethinyl estradiol concentrations in 24 volunteers following oral intake of 120 μg of ethinyl estradiol[10] *(open circles, dashed line)* and in five volunteers after vaginal administration of a 120 μg ethinyl estradiol suppository *(dots, solid line)*. All volunteers were healthy women in the early follicular phase of apparently ovulatory menstrual cycles.

Table II. Potency ratios of orally administered ethinyl estradiol as compared to ethinyl estradiol administered as vaginal polyethylene glycol suppositories with regard to gonadotropin suppression and hepatic effects

Parameters	*Ratios*
Serum FSH	4.4 : 1
Serum LH	3.2 : 1
SHBG-BC	3.5 : 1
CBG-BC	5.0 : 1
High-density lipoprotein cholesterol	4.2 : 1
Low-density lipoprotein cholesterol	4.2 : 1

after vaginal ethinyl estradiol administration diminished the discrepancy between the higher bioavailability of oral ethinyl estradiol and the lower bioavailability of vaginal ethinyl estradiol, which was mainly the result of the much lower peak ethinyl estradiol levels after vaginal administration. In view of our finding that oral ethinyl estradiol achieved peak serum levels four times as high as those obtained with ethinyl estradiol containing polyethylene glycol suppositories, it was decided to administer polyethylene glycol suppositories containing doses of ethinyl estradiol that were fourfold to fivefold as large as the 5 and 10 μg oral doses of ethinyl estradiol although the bioavailability ratio of oral-vaginal ethinyl estradiol was found to be 2.7 : 1. This decision proved to be advantageous during the second part of this study, which revealed that three to five times as much ethinyl estradiol was required to achieve the same effect on circulating FSH, LH, SHBG-BC, and CBG-BC as well as high-density lipoprotein and low-density lipoprotein cholesterol levels if given as a vaginal polyethylene glycol suppository than if ingested.

The significant decrease in circulating gonadotropins as well as the rise in SHBG-BC and CBG-BC achieved with as little as 10 and even 5 μg of oral ethinyl estradiol per day is consistent with recent literature reports.[1,4] Bolton et al.[15] as well as Robyn and Vekemans[16] presented data which indicate that 20 and 25 μg doses of oral ethinyl estradiol suppressed LH and FSH plus LH in gonadectomized and postmenopausal women, respectively. The most recent study by Mandel et al.[4] demonstrates that as little as 5 to 10 μg of daily oral ethinyl estradiol would be sufficient as an estrogen replacement dose for postmenopausal women. However, as previously noted,[1,4] even such small doses of ethinyl estradiol exert significant hepatic effects. Serum SHBG-BC is an exquisitely sensitive indicator of hepatocellular estrogen action and is more sensitive than CBG-BC.[1,4] Less sensitive indicators are plasma high-density lipoprotein and low-density lipoprotein cholesterol, previously reported to be altered by oral doses of 20 and 50 μg of ethinyl estradiol,[2,17] and plasma triglycerides, which did not show any significant response to the doses used in this study. Serum CBG-BC was found to increase in a linear dose-response curve following ingestion of ethinyl estradiol alone or in combination with progestational agents and has been used as an in vivo test for "estrogenicity" of a compound.[18] In reality, the SHBG-BC as well as CBG-BC, high-density lipoprotein and low-density lipoprotein cholesterol, and other hepatic responses to any estrogen are merely a measure of its hepatic effect and not directly related to other estrogenic effects such as gonadotropin suppression, stimulation of cell growth in estrogen-dependent tissues, and retardation of bone loss.

Whereas estrogen replacement ameliorates the disturbances of menopause such as vasomotor flushes, atrophic vaginitis, and, most important, osteoporosis, it may also be associated with liver-mediated side effects such as hypertension, thrombosis, and cholelithiasis in a manner that is dependent upon the dose and type of estrogen administered.[1-4] It has been surmised that the oral route of administration may accentuate the hepatic effects of estrogens because of the so-called first-pass effect and that the vaginal route may lessen such hepatic effects. However, the data obtained in this study indicate that vaginal administration of ethinyl estradiol, which, together with diethylstilbestrol, has a fourfold to eighteenfold higher hepatic than FSH suppressive potency as compared to natural estrogens,[1] does not selectively reduce its hepatic effects. The oral doses of 5 and 10 μg of ethinyl estradiol given daily for 25 days were appropriately chosen for estrogen replacement in postmenopausal women[4] and the vaginal doses of 20 and 50 μg of ethinyl estradiol matched the former ones with regard to bioavailability and magnitude of effects upon serum LH, FSH, SHBG-BC, and CBG-BC as well as high-density lipoprotein and low-density lipoprotein cholesterol. The dose-response curves for the pairs of lower and higher oral and vaginal doses of both FSH and SHBG-BC were parallel and permitted calculation of oral-vaginal ethinyl estradiol potency ratios of 4.4 and 3.5, respectively, demonstrating that the vaginal route of administration is not associated with a lower hepatocellular effect of ethinyl estradiol. The lack of parallelism between the dose-response curves for LH, CBG-BC, and high-density lipoprotein and low-density lipoprotein cholesterol is not surprising because only two oral and two vaginal doses were tested which precluded calculating similar oral-vaginal ethinyl estradiol potency ratios. However, the rises in LH, CBG-BC, and high-density lipoprotein cholesterol and decreases in low-density lipoprotein cholesterol observed after the lower and higher oral and vaginal doses were similar in magnitude so that approximate potency ratio estimates could be made. These potency ratio estimates in Table II indicate that vaginally administered ethinyl estradiol does not exert a lesser hepatic effect than oral ethinyl estradiol when compared by FSH and LH suppression.

Our conclusion that vaginal administration of ethinyl estradiol does not selectively reduce its hepatic effects is consistent with a recent study by Dickson and Eisenfeld.[19] These authors demonstrated with isolated rat liver parenchymal cells (1) that an approximately 100-fold higher 17β-estradiol than ethinyl estradiol concentration was required to promote nuclear translocation of hepatic estrogen receptors and (2) that tritiated 17β-estradiol was metabolized much more rapidly by the hepatocytes than was ethinyl estradiol. Thus it appears reasonable to conclude that the selectively enhanced hepatic effects of ethinyl estradiol as compared to those of other estrogens except for diethylstilbestrol[1] must be attributed to differences in hepatocellular receptor interactions and metabolism found in the 17α-ethinyl group rather than to differences with the oral route of administration. The results of this study indicate that undesirable side effects due to hepatic alterations (such as hypertension) are most likely a result of the type and dosage of estrogen administered and not of the route of administration. Administration of ethinyl estradiol by routes other than ingestion therefore does not appear to be advantageous and other estrogen formulations should be used for estrogen replacement.

The expert technical assistance of Mrs. Mary M. Francis-Hernandez and Ms. Claire Osborn is acknowledged. We thank Dr. C. E. Cook, Research Triangle Institute, Research Triangle Park, North Carolina, for a generous gift of anti–ethinyl estradiol serum where it was prepared under a contract with the National Institute of General Medicine Sciences.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. C. DONALD CHRISTIAN, Tucson, Arizona. If hallmarks of an important contribution include thoughtful experimental design, good technology, and appropriate and supportive literature citations that address a question of academic interest and great practical value and then prompt the correct conclusions, this report is such a contribution.

The question of whether the "first-pass" effect of orally administered estrogen is what evokes the marked hepatocytic protein synthesis or whether it is a result of the 17α-ethinyl substitution in ethinyl estradiol has been approached in a clever manner. The avoidance of the "first-pass" effect was accomplished by using the vaginal mucosal absorption of estrogen. This was approached first by finding the potency ratio of orally administered to vaginally administered ethinyl estradiol. By a variety of hepatic indicators (SHBG-BC, CBG-BC, and high-density lipoprotein and low-density lipoprotein cholesterol) and central nervous system indicators (LH and FSH) the potency ratio of oral-vaginal administration is rather consistently between 3 and 5. This establishment of a bioequivalency dose for the two routes of administration is in and of itself a contribution. By all indicators the equivalent oral and vaginal doses evoked the same hepatic impact, thus indicating that the substituted 17α-compound is responsible rather than the "first-pass" effect as has been postulated by some. Although it can be said that all the presented evidence is indirect it is corroborated strongly by the isolated hepatic cell studies of Dickson and Eisenfeld which is direct evidence.

The conclusion that under normal circumstances ethinyl estradiol administration by the vaginal route has no advantages for estrogen replacement and the conclusion that, because of possible undesirable hepatic effects, estrogen other than ethinyl estradiol formulations should be used for replacement both seem appropriate and clear.

As a very practical matter then, and because of the possible untoward endometrial effects of estrone preparations, it would be of interest to know which estrogen preparations the presenters think most appropriate for replacement therapy.

DR. HOWARD L. JUDD, Los Angeles, California. Dr. Mishell, in your conclusions I think you have shown that the problems in regard to ethinyl estradiol cannot be avoided by using nonoral routes of administration.

However, in one of your conclusions you indicated that the hepatic effects, apparently of all estrogens, are not route related and, if that was indeed your conclusion, then I would like to comment on that. For other estrogens it is very clearly important. There are now preparations that you are aware of and that we and others have been working with in which one can administer estrogens by nonoral routes with no hepatic effects and yet retain all of the beneficial effects desired from estrogen replacement.

Thus, for ethinyl estradiol, you have very clearly shown that the effect is probably structurally related but for other estrogens I think route is an important issue.

DR. KENNETH J. RYAN, Boston, Massachusetts. Perhaps you would comment on the possibility that some of the hepatic effects of estrogens are desirable with respect to cardiovascular disease. Elevating high-density lipoprotein cholesterol and lowering low-density lipoprotein cholesterol should be protective for women, and that is one of the conundrums we have with the use of these hormones.

DR. MISHELL (Closing). First of all, Dr. Judd, I did not mean to imply that the route of administration did not change the hepatic effects of estrogens other than ethinyl estradiol, which is all this study reported. I am aware of your work with transcutaneous administration of estradiol, and I think it shows great promise and should be pursued.

Dr. Ryan introduced a very important topic, which is the fact that there is now evidence that oral administration of conjugated equine estrogens alters certain lipid fractions in a beneficial manner. Estrogen administered to postmenopausal women can raise high-density lipoprotein cholesterol and lower low-density lipoprotein cholesterol.[1] Since high-density lipoprotein cholesterol is thought to be beneficial in preventing atherosclerosis and low-density lipoprotein cholesterol is thought to be detrimental in causing atherosclerosis, the alterations in these lipid fractions are potentially beneficial. This work was done by the lipid research group of the National Institutes of Health and was published in *The New England Journal of Medicine* last year.[1]

Furthermore, this same group has shown that patients receiving estrogen postmenopausally have a lesser chance of dying than age-matched control subjects in a given year.[2] The epidemiologic group at the University of Southern California has shown, in a case-control study of a group of women in a retirement community, that women receiving estrogen are less than half as likely to die of heart attack as control subjects who are not receiving estrogen, which is of great importance.[3] I am glad that Dr. Ryan brought this subject up because when administering estrogen one does not want to eliminate this beneficial effect on the serum lipids. Hopefully, it will be possible to eliminate the adverse effects on the globulins without altering the beneficial effects on the lipids because these are two different hepatic effects.

I want to thank Dr. Christian especially for his kind remarks. He posed a question concerning the best way to replace estrogen for postmenopausal women to avoid the proliferative effects on the endometrium, which are attributed by some to be greater with estrone sulfate than with other estrogens.

However, it is my belief that, as far as the effects on the endometrium are concerned, the different estrogens do not exert qualitative differences, only quantitative differences. Therefore, one can administer estrone sulfate and have the same endometrial effect as administering estradiol by itself, although the effect might be different quantitatively and can lead, therefore, to hyperplasia and also to adenocarcinoma by increasing steroid receptor synthesis in the endometrium. However, as we all know, this receptor synthesis can be reversed by administering progestational agents which is now common practice in the United States. The only problem that we have is which agent to administer as well as the dosage and the duration. Unfortunately, there has been very little research going on in this area, but I am hopeful that such research will be initiated in the near future so that clinicians can know the best estrogen and best progestin to administer to postmenopausal women as well as the correct dosages and the duration of each formulation every month.

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Dynamics of human chorionic gonadotropin, prolactin, and growth hormone in serum and amniotic fluid throughout normal human pregnancy

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This study was performed to establish the dynamics of human chorionic gonadotropin, prolactin, and growth hormone throughout pregnancy in serum and amniotic fluid. Two hundred fifty healthy women at 8 to 42 weeks' gestation were studied. The highest serum human chorionic gonadotropin level was measured between weeks 8 to 12 (53,715 ± 3574 mIU/ml, mean ± SEM), with a decline to a mean plateau of 11,806 ± 1250 mIU/ml from week 18. Amniotic fluid human chorionic gonadotropin had a similar pattern with a mean of 68,100 ± 8422 mIU/ml at weeks 8 to 10, declining from week 18 to a plateau of 2005 ± 260 mIU/ml. Human chorionic gonadotropin showed a significant correlation ($r = 0.85$, $p < 0.001$) between levels of both compartments demonstrating an even distribution. Prolactin levels showed a dichotomy of patterns and levels. Serum prolactin showed a continuous rise from 45.3 ± 14 ng/ml at week 8 to 224 ± 20 ng/ml at week 36. In contrast, amniotic fluid prolactin remained low until week 14 (33.1 ± 0.8 ng/ml), followed by a sharp and significant ($p < 0.001$) increase to a plateau of 3750 ± 200 ng/ml between weeks 18 to 26, declining to a second plateau of 500 ± 50 ng/ml at week 36. Serum growth hormone increased from a mean of 3.5 ± 1.4 ng/ml seen at weeks 8 to 10 to a mean of 14 ± 2.0 ng/ml at weeks 28 to 30, followed by a plateau of similar levels. The pattern of growth hormone secretion in amniotic fluid demonstrated a sharp increase during the 14-16 interval with a maximum mean level of 15.5 ± 1.5 ng/ml and a slow steady decline thereafter. In conclusion, the similar pattern and concentration of human chorionic gonadotropin throughout pregnancy in both maternal and amniotic fluid are probably the result of direct human chorionic gonadotropin diffusion from the placenta. The dissimilar pattern and concentration of prolactin are the result of two different sources of prolactin secretion during pregnancy. Serum prolactin originates from the pituitary and amniotic fluid prolactin from the decidua. Since the pattern of growth hormone secretion resembles that of prolactin, it is possible that growth hormone, like prolactin, is secreted by the same sources. (Am J Obstet Gynecol 1985;151:878-84.)

Key words: Prolactin, growth hormone, human chorionic gonadotropin throughout pregnancy

Following the initiation of pregnancy a new functional integrated endocrine system is developed. Influenced by the new steroid milieu, the maternal pituitary undergoes hyperplasia and hypertrophy of the lactotropes. Thus maternal concentration of prolactin in blood increases throughout pregnancy, while levels of growth hormone remain low and levels of luteinizing hormone and follicle-stimulating hormone become undetectable.[1-2] Neural control of the fetal pituitary is initiated by 10 to 12 weeks' gestation, as demonstrated by the detection of monoamines in the fetal hypothalamus.[3] Consequently, fetal prolactin, growth hormone, and gonadotropins are synthesized and secreted by the fetus in increasing concentrations as the duration of pregnancy lengthens.[4] The development of the placenta into a functional organ and the increased secretion of amniotic fluid into the amniotic cavity create a new compartment within the fetomaternal unit. The increase in amniotic fluid volume with advancing pregnancy, the developmental changes in fetal skin permeability, and the increase in fetal renal function add new dimensions to the intercompartmental relationship between prolactin, growth hormone, and human chorionic gonadotropin secretion. The identification of lactogenic receptors in the human chorion, and possibly in the placenta, indicates a physiologic role for prolactin and growth hormone.[5] Furthermore, it has been reported that a defect in the binding of lactogenic hormones (growth hormone and prolactin) to the chorion laeve exists in pregnancies with idiopathic polyhydramnios.[6] It has also been suggested that the extremely high

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levels of prolactin in amniotic fluid are of importance for the preservation of amniotic fluid volume and osmolarity.[7] In addition to being produced by the pituitary, maternal prolactin is also produced by the decidua.[8, 9] In order for prolactin from the latter source to be transported into the amniotic fluid, the cellular attachment between maternal decidua and fetal chorion needs to be preserved.[10] Levels of each of these hormones have been measured separately in amniotic fluid samples. To our knowledge, they have not been measured concomitantly in both serum and amniotic fluid throughout gestation. Therefore this study was undertaken in an attempt to establish the dynamics of human chorionic gonadotropin, prolactin, and growth hormone throughout pregnancy in serum and amniotic fluid.

Material and methods

The study group consisted of 250 unselected healthy women at various stages of gestation. They were not taking any medication known to affect the secretion of human chorionic gonadotropin, prolactin, or growth hormone. Gestational age was established by the date of last menstrual period, the uterine size as determined by bimanual pelvic examination or fundal height, and in almost all women by ultrasound β-scan. Those patients in whom these three parameters did not correlate were eliminated from the study. From each subject a single venous blood sample and a single amniotic fluid sample were obtained within a 15-minute time interval. Thus a total of 250 blood and 250 amniotic fluid samples were obtained from women whose gestational age, arbitrarily determined as weeks from onset of last menstrual period, varied from 8 to 42 weeks. The amniotic fluid was obtained through a 16-gauge needle introduced transcervically at the time of cervical dilatation for an elective termination of a normal pregnancy for subjects between 8 and 14 weeks of gestation (n = 26). The amniotic fluid specimens from subjects beyond 14 weeks of gestation (n = 224) were obtained by transabdominal amniocentesis with a 22-gauge needle. The clinical indications for performing the amniocentesis were elective induction of pregnancy termination with intrauterine saline solution, genetic counseling, and determination of fetal lung maturity. In this study only samples obtained from women with normal pregnancies that were terminated prematurely or who were delivered of normal fetuses were included. All of these patients receiving an amniocentesis for fetal lung maturity included in the study did not start labor for at least 48 hours after the amniotic fluid sample was obtained. All blood samples were centrifuged, the serum separated, frozen, and stored at −20° C until assayed. Measurement of human chorionic gonadotropin, prolactin, and growth hormone in amniotic fluid was performed prior to and following centrifugation in the first 30 samples measured. Since the levels of each of these hormones were identical in the two aliquots, centrifugation and supernatant separation were no longer performed. All samples of amniotic fluid were immediately frozen and stored at −20° C until assayed. Each of the three hormones were measured by radioimmunoassay. The iodinations were performed by the Chloramine T method and the free iodine separated in a Sephadex G-75 column (1 × 15 cm for human chorionic gonadotropin and 1 × 45 cm for prolactin and growth hormone). The antibody for human chorionic gonadotropin was specific for the β-subunit, recognizing it in both the free and whole molecule form. The sensitivity of the β-subunit of human chorionic gonadotropin assay was 1.5 mIU/ml, and the intra-assay and interassay coefficients of variation were 6.3% and 9.5% respectively. The sensitivity of the prolactin assay was 2 ng/ml and the intra-assay and interassay coefficients of variation were 7.0% and 9.8%. With both the human chorionic gonadotropin and prolactin assay parallelism with the unknown samples was demonstrated. The sensitivity of the growth hormone assay was 0.4 ng/ml and the intra-assay and interassay coefficients of variation were 5.8% and 10.5%, respectively. There was no appreciable cross-reaction with growth hormone and human placental lactogen in the prolactin assay or with prolactin and human placental lactogen in the growth hormone assay. The radioimmunoassay for growth hormone revealed that both serum and amniotic fluid unknown samples lacked parallelism when measured in dilutions and compared to the purified pituitary standard. It was found that the use of a different antibody (RSL-R-771) resulted in acceptable parallelism if a dilution of at least 1:2 was used. Thus all serum and amniotic fluid samples were measured with the latter antibody utilized in 1:2 and 1:4 dilutions. All samples gave parallel results in these two dilutions. Data were analyzed in a Clinfo computer system. Following logarithmic transformation of the data the statistical evaluation was performed by analysis of variance and multiple *t* test that was corrected for multiple comparisons with an overall significant level of $p < 0.05$.

Results

The number of samples obtained during each 2-week interval of gestation ranged from 6 to 50 (Table I).

The concentration of β-subunit of human chorionic gonadotropin in serum and amniotic fluid had a similar pattern in both compartments (Fig. 1). Maximum human chorionic gonadotropin values in both compartments occurred between 8 to 12 weeks and were followed by a rapid decline in the next 2 weeks. Following week 18 human chorionic gonadotropin levels in serum and amniotic fluid reached a plateau with very small

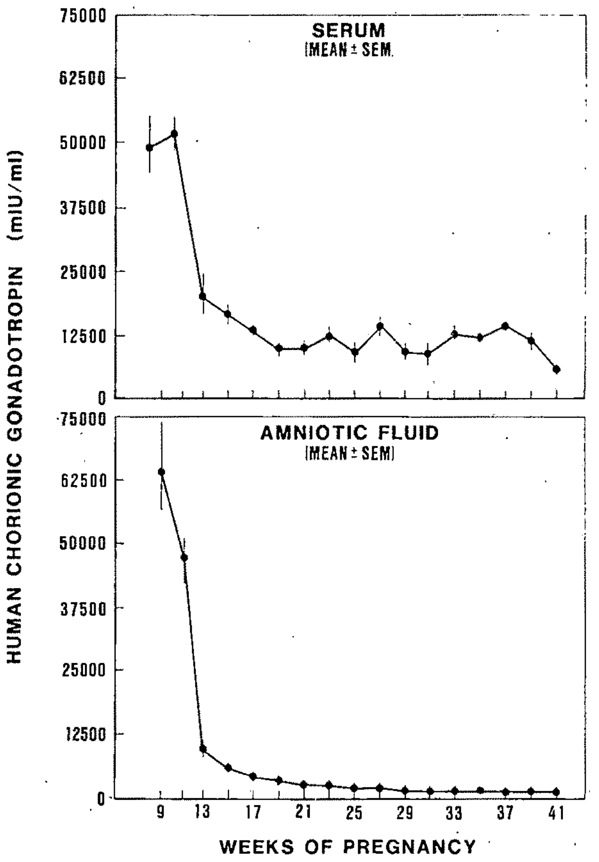

Fig. 1. Mean ± SEM concentration of human chorionic gonadotropin in maternal serum *(upper panel)* and in amniotic fluid *(lower panel)* throughout pregnancy.

variability. The initial mean ± SEM human chorionic gonadotropin concentration in serum (53,715 ± 3574 mIU/ml) was similar to that measured in amniotic fluid (68,100 ± 8422 mIU/ml). However, during the plateau period the mean ± SEM of human chorionic gonadotropin in serum (11,806 ± 1250 mIU/ml) was significantly greater ($p < 0.01$) than the mean in amniotic fluid (2005 ± 260 mIU/ml). There was a significant correlation ($r = 0.85$, $p < 0.001$) between human chorionic gonadotropin levels in both compartments.

The pattern as well as the concentration of prolactin in serum was different from that in amniotic fluid (Fig. 2). In serum there was a steady and almost linear increase of prolactin up to the 36- to 38-week interval to a maximum mean value of 224 ± 20 ng/ml ($p < 0.001$). This peak level was followed by a decline in the following 4 weeks, which became significantly less ($p < 0.002$) in the 40- to 42-week interval (130 ± 10 ng/ml). In contrast to the pattern of serum prolactin levels, amniotic fluid prolactin levels remained low until week 14 (33.1 ± 0.8 ng/ml) followed by a sharp and significant ($p < 0.001$) increase to a maximum mean value of 3750 ± 200 ng/ml during the 18- to 20-week interval. Mean levels then plateaued until 26 to 28 weeks, after which there was a steady and significant decline to a mean value of 500 ± 50 ng/ml at 34 weeks of pregnancy. Mean level plateaued again until 42 weeks of gestation.

Table I. Number of serum and amniotic fluid samples obtained at each 2-week interval of gestation based on time since onset of last menstrual period

Weeks' gestation	*n*
8-10	6
10-12	13
12-14	7
14-16	19
16-18	50
18-20	34
20-22	17
22-24	10
24-26	9
26-28	9
28-30	8
30-32	7
32-34	7
34-36	8
36-38	15
38-40	12
40-42	19

Baseline concentrations of growth hormone in serum and amniotic fluid in the first trimester were found to be within the levels seen in the nonpregnant state (0.5 to 7.5 ng/ml) (Fig. 3). However, the pattern of growth hormone levels in serum during later gestation was different from that of amniotic fluid. In serum there was a slow increase from the mean of 3.5 ± 1.4 ng/ml seen at 8 to 10 weeks to a mean of 14 ± 2.0 ng/ml at 28 to 30 weeks followed by a plateau of similar levels. There was a greater variability of values in the second half of pregnancy. The pattern of growth hormone secretion in amniotic fluid demonstrated a sharp increase during the 14- to 16-week interval with a maximum mean level of 15.5 ± 1.5 ng/ml, with a slow, steady decline thereafter. This decline in mean levels became significantly less ($p < 0.01$) during the 28- to 30-week interval and reached baseline (8- to 10-week) levels after 36 weeks of gestation.

Comment

This study demonstrates that there is a similar qualitative and quantitative pattern of human chorionic gonadotropin secretion between maternal serum and amniotic fluid throughout pregnancy. The significant correlation ($r = 0.85$, $p < 0.001$) between the levels found in both compartments indicates that the secretion of human chorionic gonadotropin from the syncytiotrophoblast is evenly distributed. Since it has been found that the levels of human chorionic gonadotropin in cord blood are extremely low,[11] it can be suggested that

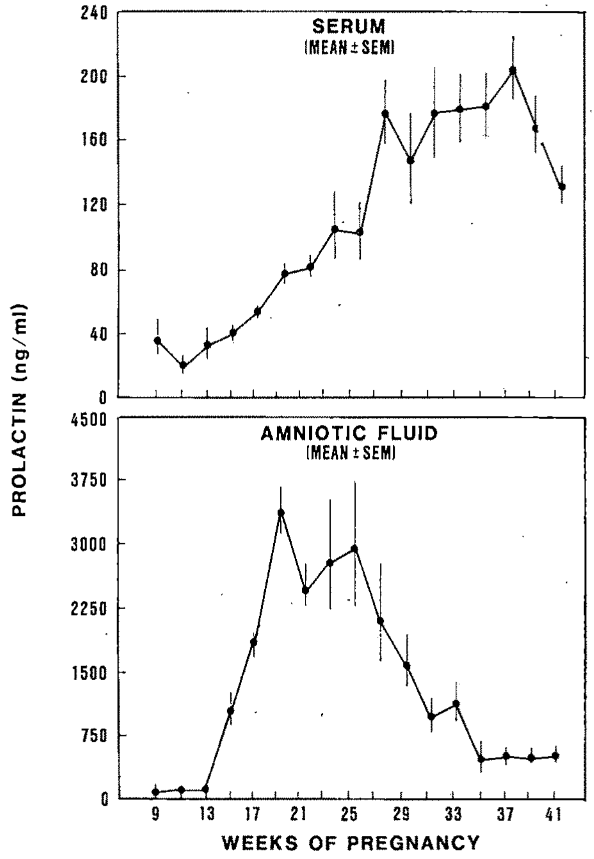

Fig. 2. Mean ± SEM concentrations of human prolactin in maternal serum *(upper panel)* and in amniotic fluid *(lower panel)* throughout pregnancy.

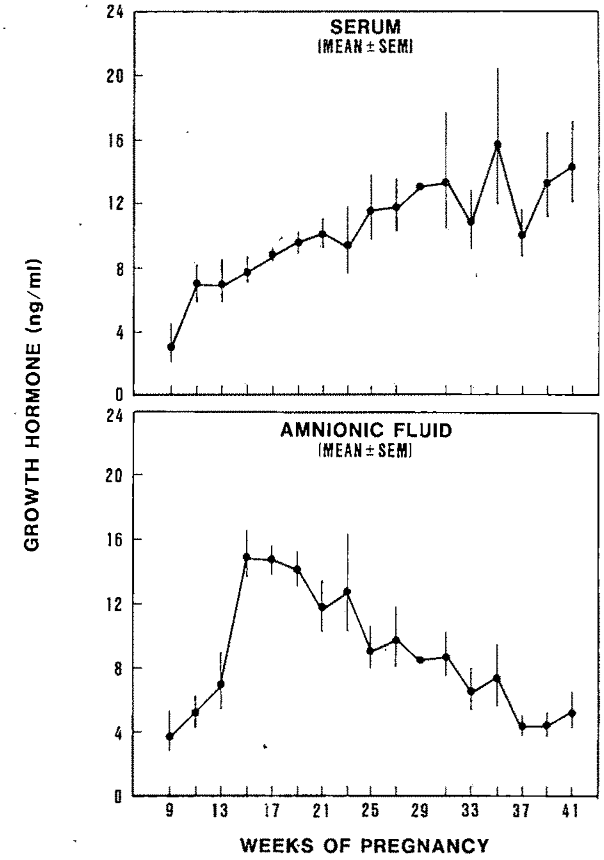

Fig. 3. Mean ± SEM concentrations of human growth hormone in maternal *(upper panel)* and in amniotic fluid *(lower panel)* throughout pregnancy.

its presence in amniotic fluid in concentrations similar to that of serum is the result of direct diffusion from the placenta. A possible reason for the fall in human chorionic gonadotropin at the end of the first 12 weeks is that the capacity of the syncytiotrophoblast to secrete human chorionic gonadotropin selectivity diminishes at that time, since it has been reported that both human placental lactogen and pregnancy-specific β-glycoprotein, which are also secreted by the same cell, continue to be secreted in high concentrations throughout pregnancy.[12] The difference in serum versus amniotic fluid concentration after week 18 could be the result of a dilutional effect produced by increasing amniotic fluid volume. The pattern of β-subunit of human chorionic gonadotropin secretion throughout pregnancy in serum is in agreement with other reports;[13, 14] however, to our knowledge the pattern of β-subunit of human chorionic gonadotropin in amniotic fluid throughout gestation has not been previously reported.

The dichotomy of prolactin patterns and concentration observed in serum and amniotic fluid throughout pregnancy is in agreement with previous reports.[1, 9] It is apparent that this disparity is the result of two different sources of prolactin secretion during pregnancy. Nearly all maternal serum prolactin originates in the maternal pituitary gland. Persistently increasing levels of estrogen and progesterone during pregnancy produce lactotrope hyperplasia and hypertrophy with a linear increase in prolactin secretion. It appears possible that this lactotrope hyperfunction reaches a peak at 36 to 38 weeks of gestation with a subsequent diminished secretion (Fig. 2). It is now apparent that the prolactin present in the amniotic fluid is secreted by the decidua capsularis,[8, 9] which most likely is not controlled by the dopamine mechanism that controls pituitary prolactin production. From this origin, prolactin crosses the chorion and amnion into the amniotic cavity as long as the anatomic integrity between decidua, chorion, and amnion is maintained.[10] However, the crossing of amniotic fluid prolactin in the opposite direction is minimal.[7] It is thought that the purpose of increased maternal prolactin is the preparation of the maternal breast for postpartum lactation. Attempts to understand the purpose of considerable higher concentrations of prolactin in amniotic fluid have been extensively investigated in different species.[15] Of all the possible functions mentioned, the most plausible is that prolactin actively participates in the control of amniotic fluid volume and osmolarity. By regulating amniotic fluid volume the high levels of prolactin could prevent sudden changes in volume and thus protect the fetus.

It has been reported that there is a doubling in the mean weekly amniotic fluid volume between 10 to 14 weeks to 14 to 18 weeks, which corresponds with the dramatic increase in amniotic fluid prolactin concentration that peaks at 18 to 20 weeks of pregnancy (Fig. 2). Similarly, fetal skin is very permeable until the seventeenth week. At this time, skin keratinization begins and the stratum corneum is completed by 25 weeks. Thus amniotic fluid could be considered to be part of the fetal extracellular space which slowly and progressively changes from week 17 to week 25 of pregnancy. At this time, prolactin concentration starts to diminish, becoming significantly lower by 30 to 32 weeks. Overlapping with the time of increase in skin keratinization is the increase in fetal renal function, at which time fetal urine becomes the most important source of amniotic fluid. The decline in amniotic fluid concentration of prolactin and growth hormone by 30 weeks could reflect the degree of maturation of fetal renal function. This is in agreement with studies demonstrating that amniotic fluid creatinine increases rapidly from 26 to 35 weeks and remains elevated until term.[16] Although several contradictory findings concerning the effect of prolactin on renal function have been reported, the presence of prolactin receptors in the proximal tubule[17] suggests that the kidneys participate in the metabolism and clearance of prolactin. Preliminary unpublished results from this laboratory indicate that prolactin is not present in urine samples obtained from infants born prematurely or at term. Thus active prolactin degradation by the fetal kidney could be an important cause in the progressive decline in amniotic fluid prolactin levels which become significantly lower by 30 to 32 weeks. Alternatively or in addition, the decline in amniotic fluid prolactin levels could represent a decreased secretion from the decidua. This view is supported by the observations made by Rosenberg et al.[9] on the in vitro decidual production of prolactin. These investigators reported that the production of prolactin by the decidua diminishes as the gestational age increases until approximately week 32.

The general pattern of growth hormone secretion in maternal serum and amniotic fluid, reported here for the first time, resembles that of prolactin. Thus, like prolactin, the maternal serum growth hormone may also be secreted by the maternal pituitary while the amniotic fluid growth hormone, like prolactin, could be secreted by the decidua. However, Bigazzi et al.[18] reported that growth hormone is not secreted in vitro by the decidua. It is probable that amniotic fluid growth hormone cannot be of maternal pituitary origin because, although the concentration is similar to that of the serum, the pattern is different. Amniotic fluid growth hormone could be of fetal pituitary origin, since a similar pattern of fetal serum growth hormone secretion with concentrations about 10-fold higher have been reported by Kaplan et al.[19] Since the amniotic fluid growth hormone concentrations are smaller than fetal serum levels, it can be suggested that amniotic fluid growth hormone does not actively participate in fetal well-being. It has been reported that the fetus has few or immature growth hormone receptors, and therefore it has been suggested that growth hormone is not essential for normal fetal growth.[19] It is presently unclear what are the specific effects (if any) of maternal and amniotic fluid growth hormone during gestation. Since it has been reported that growth hormone can induce the secretion of prolactin receptors in rat liver,[20] it is possible that the low levels of amniotic fluid growth hormone participate in the regulation of prolactin receptors. Alternatively, growth hormone may be required for the synthesis of somatomedin C, since its increase in amniotic fluid occurs after the increase in growth hormone.[16] Complete elucidation of the effects awaits further investigation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. LUTHER M. TALBERT, Chapel Hill, North Carolina. This paper presents the results of a well-designed and carefully executed study of the dynamics of chorionic gonadotrophin, prolactin, and growth hormone in maternal blood and amniotic fluid during pregnancy. This investigation is unique in that the number of patients studied is large and that values throughout pregnancy are reported. It therefore is possible to construct the striking serial curves presented by Dr. Kletzky.

The data presented are consistent with placental diffusion of human chorionic gonadotropin to both sides of the placenta into both the maternal circulation and the amniotic fluid. Serial values of prolactin and growth hormone, however, are quite different, and the values suggest that these hormones in amniotic fluid do not derive from the maternal pituitary and that placental transfer is limited. The source of growth hormone in amniotic fluid has not been identified, but Dr. Kletzky's suggestion that this hormone may derive from fetal pituitary or the decidua are reasonable hypotheses that will require further investigation. In this regard it is of interest that the year before decidual secretion of prolactin was reported,[1] several investigators commented on the great disparity of prolactin levels in amniotic fluid and fetal serum and maternal blood, and various hypotheses relating to its origin were advanced.[2,3]

There is considerable evidence that decidual prolactin may participate in the maintenance of normal amniotic fluid volumes[4] and that reduced receptor numbers for prolactin in the chorion may result in polyhydramnios.[5] I wonder whether Dr. Kletzky has speculated on the role of growth hormone in the fetus and amniotic fluid and whether he is carrying out further studies, either in vivo or in vitro, to clarify this issue. Also, in patients who are delivered near the time of collection of maternal blood and amniotic fluid, would not determination of hormone levels in fetal blood have been useful?

Finally, I very much enjoyed reviewing this excellent study. I congratulate the authors.

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DR. ROBERT B. JAFFE, San Francisco, California. I have a question and a comment.

First, I was intrigued by the lack of parallelism in one of the growth hormone radioimmunoassays. This suggests to me that perhaps there is a different form of growth hormone elaborated during pregnancy, and I would like to ask Dr. Kletzky whether he followed up on this observation.

I assume that the other two hormones assayed did demonstrate parallelism in the radioimmunoassays employed.

Second, lest you conclude that human chorionic gonadotropin measurements in the fetal compartment reflect only synthesis of human chorionic gonadotropin by the placenta, we have demonstrated relatively recently that the human fetus has the capacity to synthesize and secrete highly biologically active human chorionic gonadotropin particularly in the kidney, and this may contribute to the amount of human chorionic gonadotropin circulating in the fetal compartment.

DR. JOHN PATRICK, London, Ontario, Canada. Quite a few years ago Beverly Murphy and I performed a study similar to this on amniotic fluid cortisol concentrations during human pregnancy. Between 20 and 30 weeks' gestation the amniotic fluid samples that were available ethically tended to be ones obtained for Rh testing. The cortisols were very different in fetuses that were affected by serious Rh disease. We could use only amniotic fluids from patients that turned out to have an anamnestic Rh antibody response with fetuses that were, in fact, not affected by Rh disease. My question is, Have you separated out fetuses in the 20- to 30-week group, where these remarkable changes have occurred, that were actually not affected by Rh disease from those that had Rh isoimmunization? If this has not been done, fluids from Rh-immunized pregnancies might have influenced your time-curve data.

DR. ROBERT C. GOODLIN, Omaha, Nebraska. In our literature there are many reports that the amniotic fluid human chorionic gonadotropin is elevated in toxemia and in growth-retarded fetuses. I have wondered if this is because these women also have oligohydramnios.

Have you considered the volume of the amniotic fluid when you speak of human chorionic gonadotropin concentrations? The other issue is, and at least it is my belief, that ultrasound studies have shown that the fetal urine controls the amniotic fluid volume from 16 weeks onward, not beginning at 20 or 24 weeks as you implied.

DR. KLETZKY (Closing). As stated before, it is possible that amniotic fluid growth hormone participates in the regulation of prolactin receptors or it may be required for the synthesis of amniotic fluid somatomedin C; however, further investigation is needed. Since it is possible that amniotic fluid growth hormone may be a reflection of fetal pituitary growth hormone secretion, we are presently engaged in a study that includes pregnant women carrying anencephalic fetuses. If the source of amniotic fluid growth hormone is of fetal pituitary, the concentration of growth hormone in amniotic fluid should be very low in these pregnancies, since these fetuses have no hypothalamus. Measurement of hormones in fetal blood will be then included.

Dr. Jaffe asked about the lack of growth hormone parallelism. This was a surprising finding. Parallel studies of growth hormone were done for completion of the study. The levels of prolactin and human chorionic gonadotropin were very high and were found to be parallel to the standard. The levels of growth hormone were mildly elevated and lacked parallelism. As pointed out there exists the possibility that during pregnancy growth hormone is secreted in the form of different molecules. However, it should be remembered that the use of a different antibody rendered parallel results. Further work is being presently done. At the present time I do not have a final answer to the question.

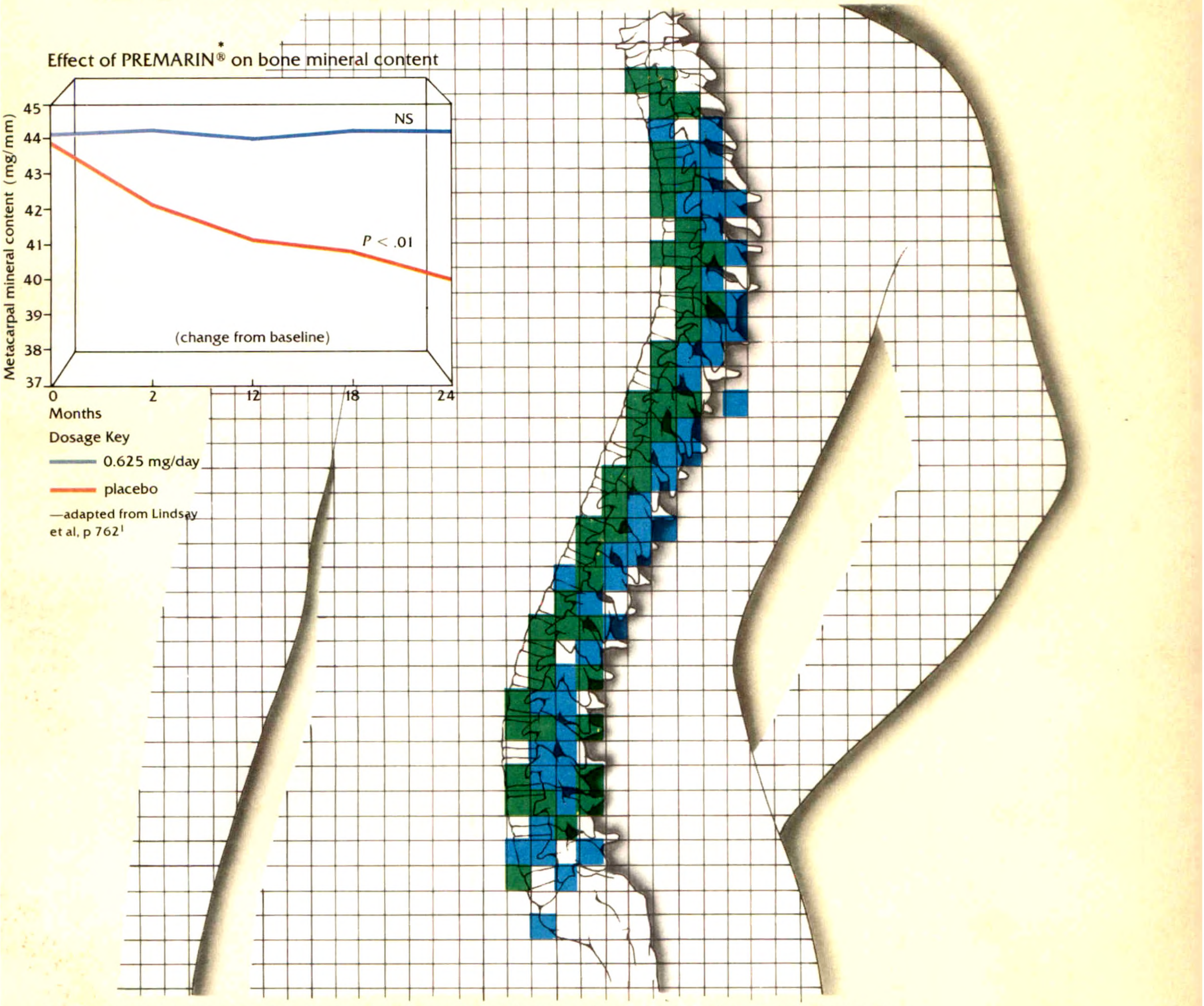
Dr. Jaffe also pointed out that human chorionic gonadotropin is secreted by the fetal kidney. Since this occurs at the time when the concentration of human chorionic gonadotropin is well below the initial high concentration seen during the first 12 weeks of pregnancy, the urine human chorionic gonadotropin may contribute to the maintenance of only the lower levels of human chorionic gonadotropin.

In regard to Dr. Patrick's question, I can say that the independent analysis of the results of those individuals studied between the weeks 20 and 30 of pregnancy revealed the same results as those presented. We should remember that our data are based on normal pregnancies and that we were not dealing with measurement of cortisol that is affected by many different stressful stimuli.

In regard to the question of human chorionic gonadotropin in toxemia, we studied about 45 additional women with toxemia, diabetes, and hypertension, and the results were compared to the 95% confidence limits for each hormone. In this preliminary study we found that values of all samples of human chorionic gonadotropin, prolactin, and growth hormone at different times of pregnancy fell within the 95% confidence limit. Of course, this is only preliminary, but this is all that I can comment on at the present time.

POSTMENOPAUSAL BONE LOSS THAT INCAPACITATES

Effect of PREMARIN® on bone mineral content*



—adapted from Lindsay et al, p 762[1]

PREMARIN MAY HALT THE DISABLING COURSE OF OSTEOPOROSIS*

Osteoporosis has an enormous epidemiological impact: it affects 10 million American women, and 26% of all women over age 60.[2] The disease begins silently and progresses inexorably for 15 to 20 years, until disabling complications occur.[3]

To minimize osteoporotic damage, the condition must be detected early and treated promptly. For many patients, PREMARIN is optimal therapy for osteoporosis, as part of a comprehensive program that includes exercise, good nutrition, and calcium supplements. In a controlled study of postmenopausal and oophorectomized women, PREMARIN (Conjugated Estrogens Tablets, U.S.P.) doses of 0.625 mg/day prevented loss of metacarpal mineral content (see graph above).

PREMARIN®

(CONJUGATED ESTROGENS TABLETS, U.S.P.)

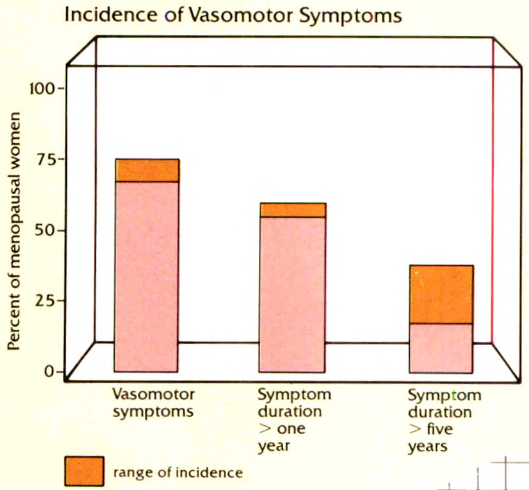
0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

The appearance of these tablets is a trademark of Ayerst Laboratories.

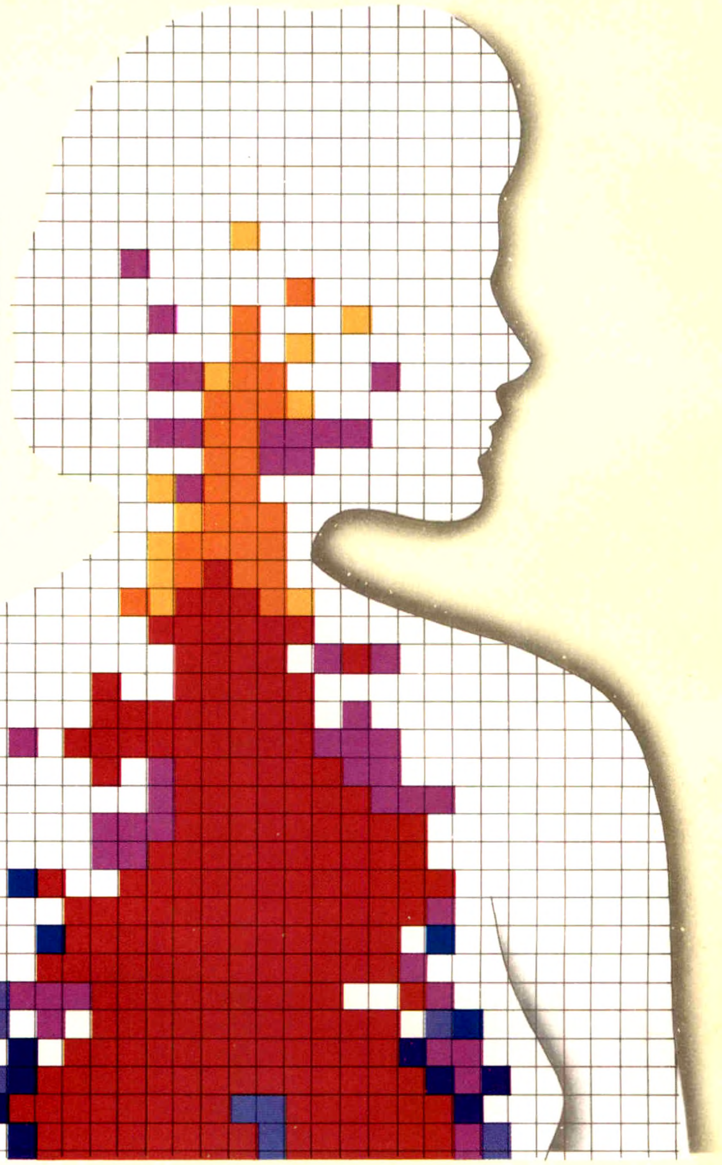
*Conjugated Estrogens Tablets have been evaluated as probably effective for estrogen-deficiency-induced osteoporosis.

Please see last page for brief summary of full prescribing information.

VASOMOTOR SYMPTOMS THAT DEMAND INTERVENTION



—adapted from Judd, p 20[4]



PREMARIN RELIEVES MODERATE TO SEVERE VASOMOTOR SYMPTOMS

Vasomotor symptoms are the most common manifestation of the menopause, affecting up to 75% of menopausal women. Of these, 80% may suffer for more than a year and up to 50% for more than five years.[4] These symptoms can disrupt a woman's life by chronically interrupting sleep, resulting in anxiety and irritability.

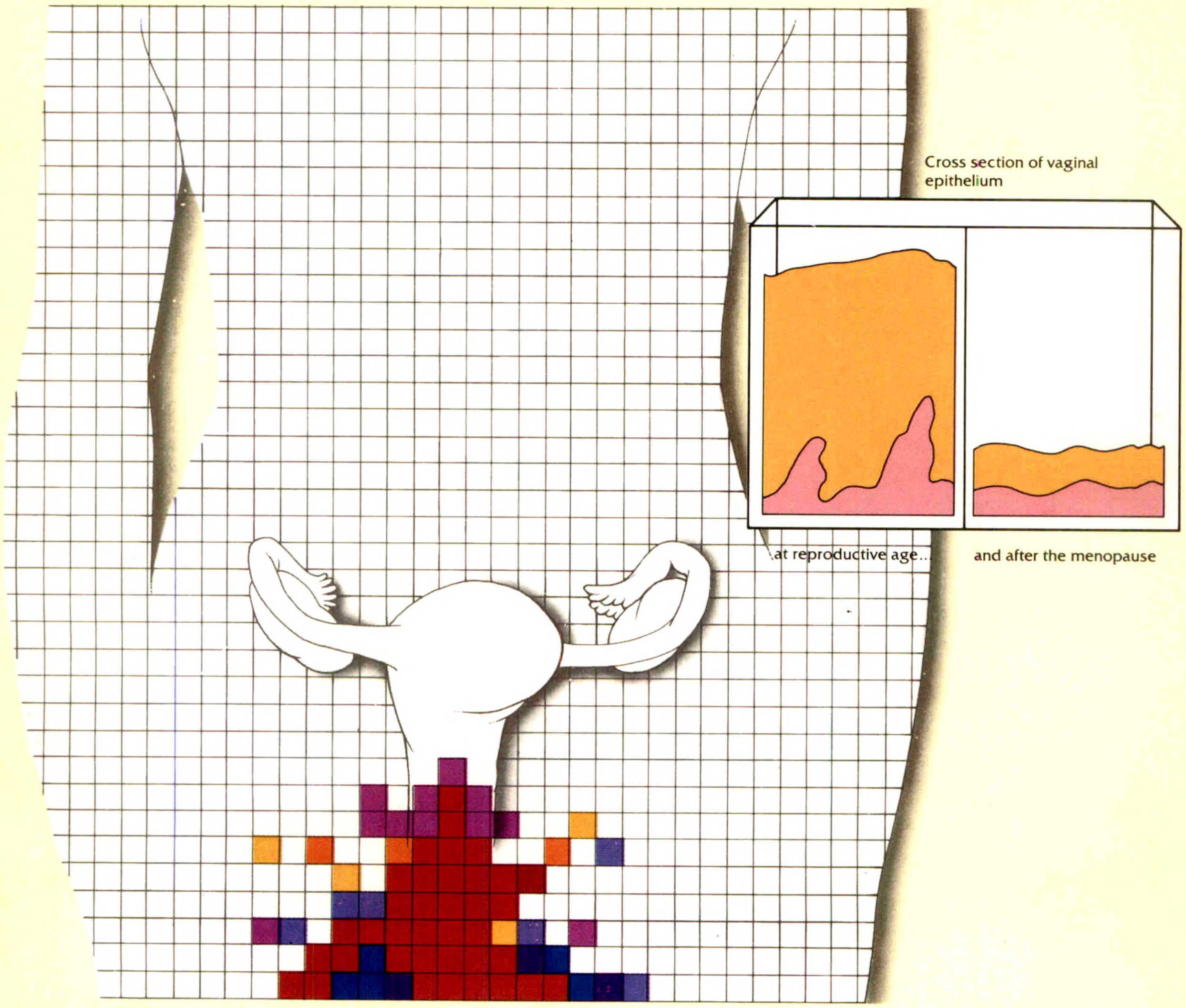
In a study of postmenopausal women suffering severe episodes of cutaneous flushing, symptoms improved markedly after administration of estrogen[5]—the treatment of choice for moderate to severe vasomotor symptoms.[6] The estrogen of choice is PREMARIN, the most widely prescribed estrogen for over 40 years. PREMARIN (Conjugated Estrogens Tablets, U.S.P.) relieves moderate to severe vasomotor symptoms of the natural menopause, as well as the acute and often severe symptoms of surgical menopause.

PREMARIN®
(CONJUGATED ESTROGENS TABLETS, U.S.P.)

The appearance of these tablets is a trademark of Ayerst Laboratories.

Please see last page for brief summary of full prescribing information.

VAGINAL ATROPHY THAT
INTERFERES WITH SEXUALITY

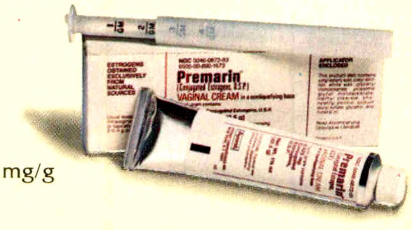

Cross section of vaginal epithelium
...at reproductive age...
and after the menopause

PREMARIN RESTORES THE VAGINAL ENVIRONMENT

In the postmenopausal woman, decreasing levels of estrogen can have devastating effects on a woman's sexual functioning. The pH of vaginal secretions rises, promoting the growth of contaminating organisms. The vaginal epithelium dries and thins, becoming susceptible to irritation, injury, and infection. Sexual relations may be difficult or impossible.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream focuses therapy at the site of the problem. Vaginal dryness is relieved, pH reverts to its normal acidity, and the epithelium thickens and becomes more resistant to injury and infection. With the vaginal environment returned to its premenopausal state, sexual function may improve.

PREMARIN®
(CONJUGATED ESTROGENS, U.S.P.) Vaginal Cream



0.625 mg/g

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Please see last page for brief summary of full prescribing information.

ACKAGE CIRCULAR)

REMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.
REMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P.) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)

2. Atrophic vaginitis
3. Kraurosis vulvae
4. Female hypogonadism
5. Female castration
6. Primary ovarian failure
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used n treatment of breast or prostatic malignancy).

WARNINGS: Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent study has raised this possibility. There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an ncreased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast have been shown to increase the risk of nonfatal myocardial infarction, thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

1. *Given cyclically for short-term use only.* For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. *Given cyclically:* Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

3. *Given for a few days:* Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. *Given chronically:* Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women—10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

Usual dosage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865—Each *purple* tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866—Each *yellow* tablet contains 1.25 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864—Each *white* tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867—Each *maroon* tablet contains 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and unit dose package of 100. No. 868—Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream—No. 872—Each gram contains 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white wax, glyceryl monostearate, propylene glycol monostearate, methyl stearate, phenylethyl alcohol, sodium lauryl sulfate, glycerin, and mineral oil.)

Combination package: Each contains Net Wt. 1½ oz. (42.5 g) tube with one calibrated plastic applicator.

Also Available—Refill package: Each contains Net Wt. 1½ oz. (42.5 g) tube. **4340R1/285**

References: 1. Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **2.** Katz WA: *Rheumatic Diseases: Diagnosis and Management*. Philadelphia, JB Lippincott Co, 1977, p 672. **3.** Reese WD: A better way to screen for osteoporosis. *Contemp Ob/Gyn* 1983;22(November):116-131. **4.** Judd HL: After the menopause. *Transition* 1983;1:19-30. **5.** Erlik Y, Tataryn IV, Meldrum DR, et al: Association of waking episodes with menopausal hot flushes. *JAMA* 1981;245:1741-1744. **6.** Meldrum DR: The pathophysiology of postmenopausal symptoms. *Sem Reprod Endocrinol* 1983;1(February):11-17.

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Uterine blood flow and catecholamine response to repetitive nicotine exposure in the pregnant ewe

Robert Resnik, M.D., Wayne B. Conover, M.D., Thomas C. Key, M.D., and Helen VanVunakis, Ph.D.

San Diego, California, and Waltham, Massachusetts

In order to simulate human smoking, experiments were designed to determine what dose of nicotine in the pregnant sheep would produce those plasma nicotine concentrations observed in human smokers and to measure uterine blood flow and plasma catecholamines in response to repetitive exposure (every 30 minutes) to that nicotine dose. Utilizing seven chronically catheterized pregnant sheep equipped with electromagnetic flow probes around both uterine arteries, we observed that a nicotine dose of 0.2 mg/min for 5 minutes results in mean plasma nicotine concentrations of 23.1 ± 1.1 ng/ml SEM ($n = 17$) immediately following infusion. This dose of nicotine was then infused every 30 minutes for 4 hours, and aliquots of blood were drawn immediately before and after nicotine infusion for determination of plasma catecholamines. No significant alterations in plasma epinephrine and norepinephrine were observed throughout the experiments ($n = 8$), and no significant changes in uterine blood flow occurred at any time during the experiment ($n = 30$). We conclude that there is a species difference between sheep and man with respect to the nicotinic threshold for catecholamine release. (AM J OBSTET GYNECOL 1985;151:885-91.)

Key words: Nicotine, uterine blood flow, catecholamines

Inhalation of cigarette smoke by pregnant women has been implicated in numerous adverse effects on fetal outcome. Reported complications include low birth weight because of prematurity and suboptimal fetal growth, antepartum bleeding, and an overall increase in perinatal mortality rate. Among the proposed mechanisms responsible for these collective observations are the decreased caloric intake reported in smokers and impairment of fetal oxygenation resulting from the presence of high concentrations of carbon monoxide in tobacco smoke.[1, 2]

Previous studies in our laboratory have demonstrated that systemic infusions of nicotine into pregnant sheep result in substantial decreases in uterine blood flow.[3] These decreases are observed within 5 to 7 minutes following the infusion of 1 mg/min for 10 minutes (14 to 32 μg/kg of body weight per minute) and are mediated by the local release of norepinephrine from adrenergic axon terminals. In support of this proposed mechanism are the combined observations of striking increases in plasma norepinephrine concentrations following exposure to nicotine, and the suppression of the response following pretreatment with the α-blocker phentolamine.[3] However, we subsequently demonstrated that nicotine infused at only 0.5 mg/min has no measurable effect on maternal or fetal cardiovascular dynamics and plasma catecholamine concentrations,[4] despite producing nicotine plasma concentrations 4 to 6 times higher than those reported in smoking humans.[5]

These findings suggest either that the experimental design of a 5-minute nicotine infusion does not adequately simulate the human smoking experience or that there is a species difference between sheep and humans with respect to the threshold for nicotine-induced catecholamine release. To resolve this question, additional experiments were designed to determine precisely what dose of nicotine produces plasma concentrations in pregnant sheep similar to those observed in smoking humans and to measure catecholamine and associated physiologic responses resulting from repetitive exposure to that nicotine dose.

Material and methods

Seven cross-bred pregnant ewes with gestational ages between 60 and 120 days were surgically prepared for study during this investigation. All food was withheld for 48 hours prior to surgery with the animals allowed access to water ad libitum. Following sedation with intravenous sodium pentobarbital, 600 mg, a spinal anesthetic (tetracaine hydrochloride, 6.7 mg) was administered. During the procedure, the subarachnoid block

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From the Department of Reproductive Medicine, University of California, San Diego, School of Medicine, and the Department of Biochemistry, Brandeis University.

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Reprint requests: Robert Resnik, M.D., University of California, San Diego Medical Center H-813, 225 Dickinson St., San Diego, CA 92103.

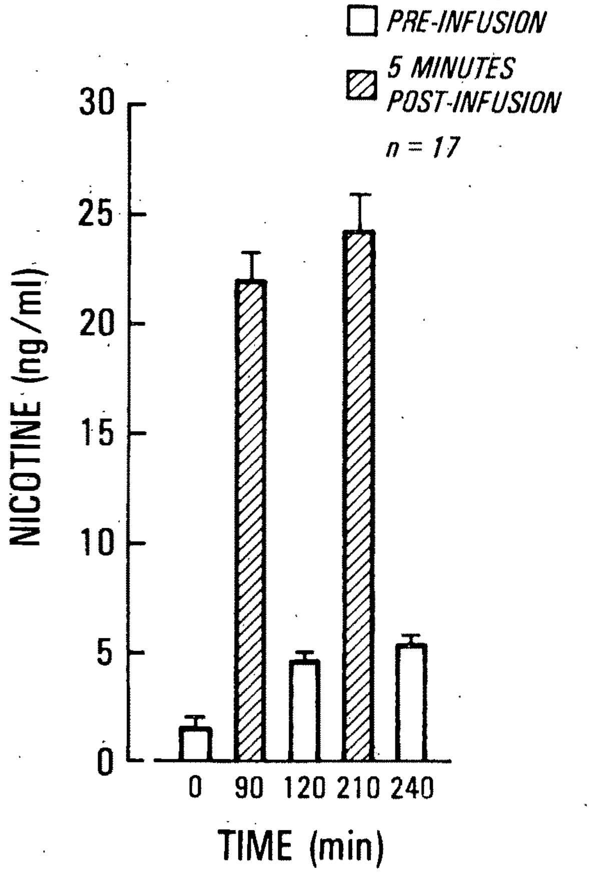

Fig. 1. Preinfusion and postinfusion plasma concentrations of nicotine (mean ± SEM) during study. Nicotine was administered at 0.2 mg/min for 5 minutes every 30 minutes.

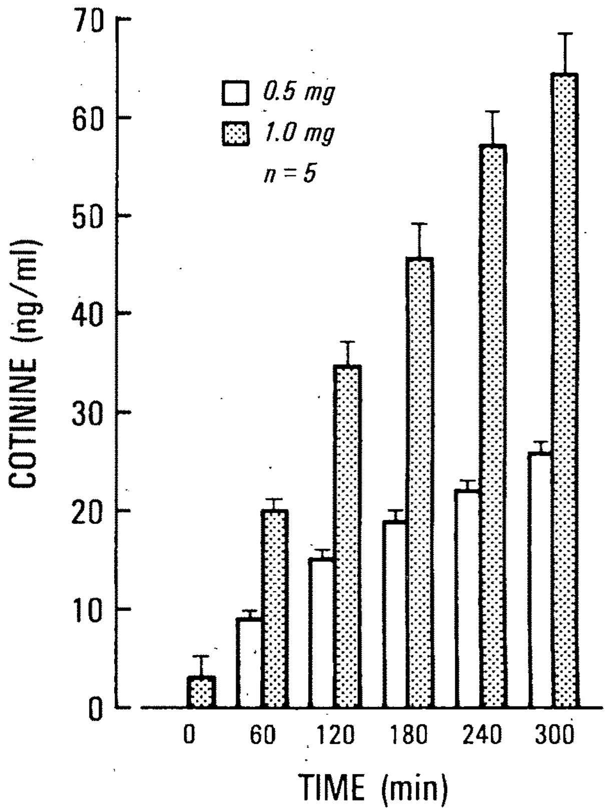

Fig. 2. Preinfusion plasma concentrations of cotinine (mean ± SEM) 30 minutes following nicotine administration of either 0.5 mg (0.1 mg/min) or 1.0 mg (0.2 mg/min). Note the accumulation of the nicotine metabolite in the circulation.

was supplemented with intravenous pentobarbital as required. Pelvic laparotomy was performed through a midline incision. A pregnant uterine horn was exteriorized and the anterior leaf of the broad ligament incised, exposing the uterine artery. A 3 or 5 mm electromagnetic flow probe (Micron Instruments, Inc., Los Angeles, California) was placed around the uterine artery proximal to its bifurcation. Aortic and inferior vena cava polyvinyl catheters (inside diameter, 0.86 mm; outside diameter, 1.52 mm) were placed via retrograde insertion through the maternal femoral artery and vein. All catheters and flow probe leads were brought through a subcutaneous tunnel to a storage pouch on the animal's flank. Catheters were flushed daily with heparinized saline solution (1000 U/ml). The details of the surgical procedure have been described in previous reports.[3, 4]

After operation the ewes were kept in a rectangular stainless steel stall, which remained in the laboratory for the duration of the studies. Experiments were begun following the demonstration of stable uterine blood flow but in no instance earlier than the fourth postoperative day. No animal was studied more than once per day or beyond 143 days of gestation. Uterine arterial blood flow was measured with an electromagnetic flow meter (Model R.C. 1000, Micron Instruments, Inc.) and displayed on a pen recorder (Model 220, Gould, Inc., Oxnard, California) running at a speed of 1 mm/min.

A nicotine solution containing 0.2 mg/ml was prepared for each animal by diluting 0.1 gm of nicotine (Eastman Kodak Company, Rochester, New York) with 0.9% saline solution. This solution was administered into the maternal inferior vena cava by a constant infusion pump (Model 940, Harvard Apparatus Company, Inc., Millis, Massachusetts).

Phase 1—Determination of plasma nicotine and cotinine concentrations following repetitive nicotine administration. Five-minute infusions of 0.1 mg/min or 0.2 mg/min of nicotine were made at 30-minute intervals for a total of 10 exposures. Only one experiment was conducted each day and all infusions were of the same dose. Aliquots of maternal blood were collected in heparinized syringes prior to the beginning of the

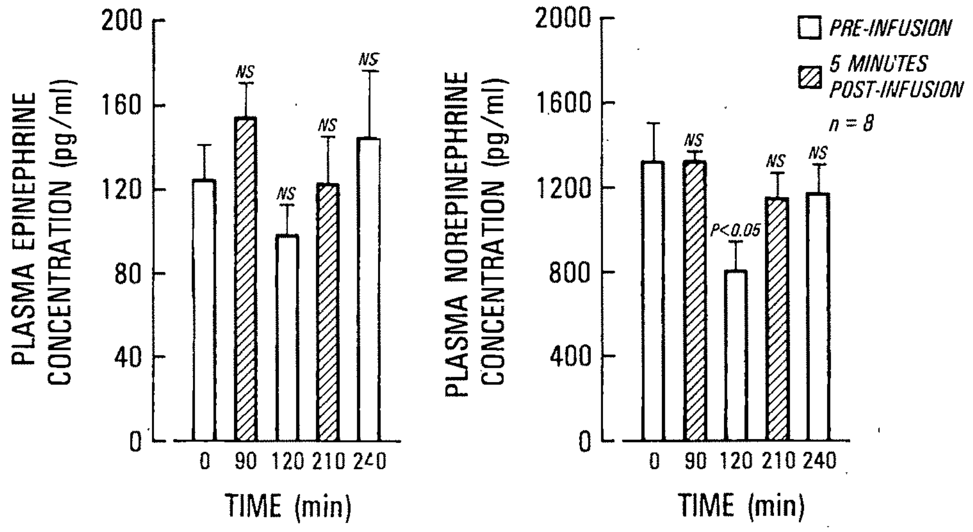

Fig. 3. Preinfusion and postinfusion plasma concentrations of epinephrine and norepinephrine (mean ± SEM) following nicotine infusion of 0.2 mg/min for 5 minutes. All concentrations are compared to that observed at time zero.

experiment, immediately following and 5 minutes prior to infusions at 90, 120, 210, and 240 minutes. All blood samples were centrifuged and the plasma frozen for analysis of nicotine and cotinine.

Phase 2—Determination of maternal physiologic responses to nicotine infusion. Nicotine doses of 0.2 mg/min (the dose observed to produce plasma nicotine concentrations in the sheep similar to that observed in smoking humans) were infused at 30-minute intervals for a total of eight doses as described in phase 1. Aliquots of maternal blood for nicotine, cotinine, and catecholamine determinations were collected in heparinized tubes at the time intervals described in phase 1. All blood samples were centrifuged and the plasma frozen for analysis at a later date. Continuous measurements of uterine blood flow, blood pressure, and heart rate were started 30 minutes prior to the experiment and continued until 30 minutes following the last infusion of nicotine.

Epinephrine and norepinephrine were measured by means of a modified single isotopic radioenzymatic assay summarized in previous reports.[3, 6] The sensitivity of the assay is 5 to 20 pg/ml. The assay is linearly related (r = 0.999) for catecholamines to at least 1 ng. The intraassay variations for epinephrine and norepinephrine are 5.9% and 4.5%, respectively; the interassay variations are 14.9% and 12.8%, respectively. Plasma nicotine and cotinine concentrations were measured by radioimmunoassay as described by Langone and VanVunakis.[7]

Plasma concentrations of nicotine, cotinine, and catecholamines are expressed as mean ± SEM. Blood flow data are expressed as percentage of change from the baseline flow just prior to each nicotine infusion. Comparison of concentrations and blood flows were made by a two-way analysis of variance, multiple range test, and Student's paired t test where appropriate.

Results

Plasma nicotine and cotinine concentrations prior to and following nicotine infusions. A nicotine infusion rate of 0.2 mg/min for 5 minutes produced a plasma nicotine concentration of 23.1 ± 1.1 ng/ml (n = 17) immediately following infusion. Five minutes prior to the next infusion, the plasma nicotine concentration averaged 4.15 ± 1.3 ng/ml. These concentrations are similar to those reported in human smokers.[5]

Plasma cotinine concentrations increased from 9.1 ± 0.3 to 25.7 ± 0.9 ng/ml at a nicotine infusion rate of 0.1 mg/min, and 19.8 ± 1.2 to 64.4 ± 4.1 ng/ml at 0.2 mg/min nicotine infusion rate. These data are summarized in Figs. 1 and 2.

Maternal physiologic and catecholamine responses to nicotine infusion. Maternal plasma epinephrine and norepinephrine concentrations were compared to the concentration observed at time zero. Maternal plasma epinephrine concentrations did not differ significantly from preinfusion values whether measured immediately following or 30 minutes after an infusion of nicotine. Maternal plasma norepinephrine showed a significant decrease from the baseline value prior to the infusion at 120 minutes. However, there were no significant increases in norepinephrine levels immediately following nicotine infusions (Fig. 3).

Analysis of the physiologic data, including uterine blood flow, heart rate, and mean arterial pressure, re-

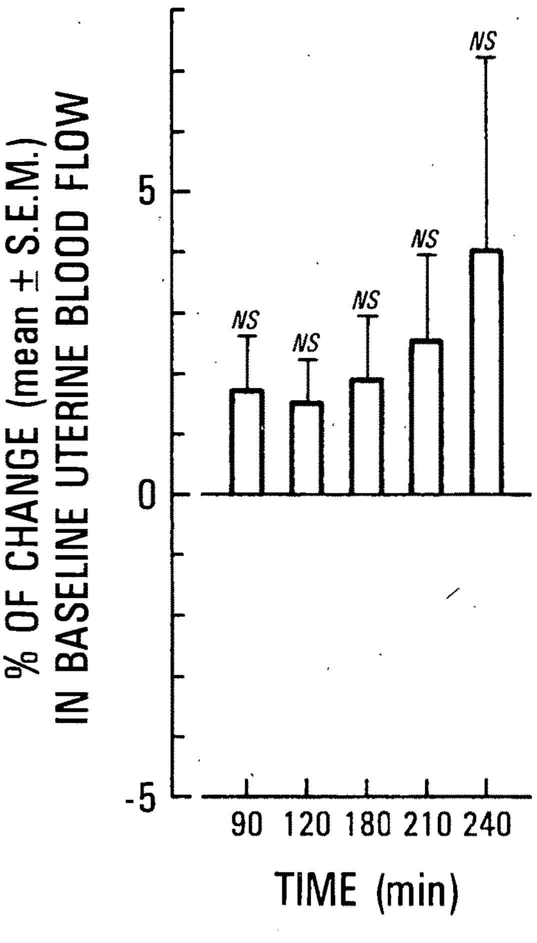

Fig. 4. Percent change in uterine blood flow following repetitive nicotine exposure.

vealed no significant changes when the initial baseline rate was compared to that following the last infusion in the experimental sequence. The uterine blood flow data are summarized in Fig. 4.

Comment

Despite repetitive nicotine exposure in pregnant sheep sufficient to produce plasma nicotine concentrations similar to those found in smoking humans, we were unable to demonstrate any significant alteration in maternal catecholamines, uterine blood flow, heart rate, or mean arterial pressure. At higher doses (1.0 to 1.5 mg/min), uterine blood flow decreases concomitant with a rise in heart rate and mean arterial pressure,[3, 4] and similar findings have been reported with large nicotine doses (100 μg/kg/min) in the pregnant rhesus monkey.[8]

Data obtained from smoking humans suggest that the release of catecholamines, as measured by increased plasma epinephrine and norepinephrine, occurs in response to the amount of nicotine in cigarette smoke. Cryer et al.[9] have reported increases in heart rate and blood pressure in men following cigarette smoking with an associated increase in plasma catecholamines. The physiologic responses they observed were blocked by phentolamine and propranolol, which is in agreement with our previously reported data in pregnant sheep,[3] and further supports the concept that the response to nicotine is mediated by the release of catecholamines. However, of particular note is the observation of Cryer et al. that heart rate and blood pressure changes occur prior to increases in plasma catecholamines, leading them to conclude that the physiologic alterations are the results of local release of norepinephrine from adrenergic axon terminals rather than increases in circulating catecholamines.

Quigley et al.[10] have demonstrated increases in blood pressure and maternal and fetal heart rate in smoking pregnant women as well as an increase in plasma catecholamines. Furthermore, it has been shown that amniotic fluid catecholamines and their metabolites are elevated in pregnant smokers.[11] Although it is not possible to record direct measurements of uterine blood flow in humans, Lehtovirta et al.[12] noted a decrease in intervillous blood flow following smoking, utilizing an intravenous xenon 133 technique. These combined observations suggest that the release of catecholamines is a prerequisite for such physiologic alterations, that the alterations occur at lower plasma nicotine concentrations, and that the threshold for adrenergic release in response to nicotine is higher in sheep than in humans.

The biologic implications of the accumulation of cotinine, a major metabolite of nicotine, which we observed in our experiments is not clear at present. Its measurement may be used as a marker of nicotine exposure since its half-life is much longer (approximately 24 hours) compared to nicotine (30 minutes).[7, 13] Nicotine and cotinine concentrations in the amniotic fluid of smokers are elevated,[14] confirming fetal exposure to nicotine in pregnant women exposed to cigarette smoke. The cotinine concentrations in passive smokers are also elevated.[15]

Current data suggest that cigarette smoking has a deleterious effect on the fetal environment and that in human smokers the response is mediated by the release of catecholamines in response to nicotine stimulation. We have reported similar physiologic alterations in sheep. However, when repetitive nicotine exposure in sheep is utilized in a manner that simulates human smoking, producing plasma nicotine concentrations similar to those in humans, no physiologic alterations occur. These variations in species threshold suggest that the development of other animal models may be required to more precisely delineate the relationship between nicotine and the fetal environment.

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Discussion

DR. CARLYLE CRENSHAW, JR., Baltimore, Maryland. The two purposes of these investigations were (1) to determine the rate of nicotine infusion into the pregnant, chronically instrumented sheep model which would produce maternal plasma nicotine concentrations equivalent to those seen in pregnant women who smoke and (2) to utilize these infusion rates to determine the effect of nicotine upon maternal pulse blood pressure, catecholamine response, and uterine blood flow.

Prior studies[1] have indicated that there is an increase in maternal blood pressure, maternal and fetal heart rate, and maternal plasma catecholamine concentration in pregnant women who smoke. It has been postulated that these physiologic responses are responsible for the decrease in fetal weight and increase in perinatal mortality rates seen in smoking mothers.

Prior studies by Resnik et al.[2] and Monheit et al.[3] have demonstrated that the systemic administration of "pharmacologic" doses of nicotine (1.0 to 1.5 mg/min for 15 minutes) to the pregnant sheep model produces an increased release of norepinephrine and epinephrine, an increase in maternal blood pressure and pulse, and a decrease in uterine blood flow.

Under the circumstances of the current investigations (i.e., infusing nicotine at a rate of 0.2 mg/min for 5 minutes every 30 minutes for a total exposure of 240 minutes and producing plasma concentrations equivalent to those seen in smoking women) no significant changes were produced in maternal heart rate and blood pressure, norepinephrine and epinephrine concentrations, or uterine blood flow.

These findings are not surprising in view of previous studies by Monheit et al.[3] indicating that a nicotine infusion of 0.5 mg/min for 15 minutes into the pregnant ewe, (producing a nicotine concentration four to six times higher than those concentrations reported in smoking humans) did not produce either alterations of maternal hemodynamics or catecholamine release.

I do have several questions for Dr. Resnik: (1) What effect (if any) does the administration of nicotine have upon estrogen and progesterone concentrations in the pregnant sheep or pregnant woman? (2) Have studies been performed in the pregnant sheep chronically infusing nicotine for weeks or months at the lower dose (0.2 mg/min to yield plasma nicotine concentrations comparable to those in smoking women) to determine if there is a deleterious effect upon neonatal outcome even though acute changes in uterine blood flow or catecholamine secretion are not demonstrable? (3) Have catecholamine turnover rates locally (i.e., in the uteroplacental unit) been determined by the tyrosine hydroxylase inhibition assay of Brodie et al.[4] in sheep after nicotine administration? This more sensitive, comprehensive analysis of catecholamine formation and turnover locally might be useful in detecting possible changes in tissue catecholamine secretion after the administration of low doses of nicotine. (4) Have the authors had the opportunity of looking at the effect of infusions of nicotine upon uterine blood flow at earlier stages in pregnancy than those reported in the present investigations?

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over time of tissue catecholamines. J Pharm Exper Therap 1966;154:493.

Dr. Frank C. Greiss, Jr., Winston-Salem, North Carolina. Despite the now ubiquitous warning that "the Surgeon General has determined that cigarette smoking is dangerous to your health," men and women continue to smoke and obstetricians will continue to encounter pregnant smokers. For those women who cannot or will not stop smoking during pregnancy, it behooves us to learn as much as possible about the mechanisms of smoking's deleterious effects on childbearing. Dr. Resnik's paper stimulated me to review again what I had remembered as somewhat chaotic literature on this subject. I find that not much has changed, in part for understandable reasons.

A study of the effects of cigarette smoking should involve cigarette smoke instead of nicotine, since there are humoral and nervous mechanisms triggered by the inhalation of cigarette smoke which can antagonize the primary effects of nicotine alone. Have you ever tried to make a sheep smoke? Many years ago Tom Kirschbaum and I independently tried to do this. It was not easy then nor is it today. Therefore studies in subhuman primates and sheep have involved nicotine infusions in highly variable concentrations, most often without determination of serum nicotine levels.

In humans, the heaviness of smoking has been measured by the number of cigarettes smoked per day, but equally pertinent factors such as nicotine content of the cigarette, puff frequency, puff velocity, butt size, and tolerance have been considered only rarely. Measurement of placental blood flow is limited to isotope clearance studies that are cumbersome, inaccurate, and theoretically if not actually dangerous enough to be banned in the United States.

Despite these problems, certain facts emerge it seems to me. (1) The effects of cigarette smoking result from the composite effects of placental blood flow reductions, decreased oxygen carrying capacity from increased levels of carboxyhemoglobin, increased blood concentrations of cyanide and thiocyanate, and possible nutritional factors either at the maternal ingestion or the placental transfer levels. (2) Despite smoking patterns, serum concentrations of nicotine are significantly reduced by low-nicotine content of the smoked cigarette. (3) If smoking does not increase the blood pressure and pulse rate, it is unlikely that placental blood flow will be affected. (4) The thresholds for nicotine-induced systemic and uterine vascular effects vary widely not only between species but within the human species. For example, in humans, smoking may induce an increase, no change, or a decrease in placental blood flow. When decreases have occurred, they have exceeded the other responses sufficiently that the mean effect has been a negative one. (5) In an otherwise normal pregnancy with adequate placental reserve, even heavy smoking will result in an infant who is 300 gm smaller than normal but otherwise healthy. The problem as always is identification of the adequacy of placental reserve. Women at high risk from other factors should stop smoking or at a minimum smoke fewer low–nicotine content cigarettes (<0.2 mg).

None of these comments are intended to denigrate the present and past contributions of Dr. Resnik and his associates to our basic knowledge in this area. They have demonstrated that the effects of nicotine are adrenergically mediated and that species variations must be considered in all such studies. More importantly, the present study is the only one I have found that is sufficiently comprehensive as to include not only systemic cardiovascular and local uterine effects but blood nicotine, cotinine, and catecholamine levels as well.

Dr. Frederick P. Zuspan, Columbus, Ohio. There is one parameter that I would like to have you consider and to ask whether this was done. Did you consider collecting maternal sheep urine since occasionally when there are only minimal increases in plasma amines, this may act as a mean collector of amines and show increases that might be significant.

Dr. Thomas Kirschbaum, Bethesda, Maryland. I want you to know we did succeed in making our sheep smoke with an indotracheal tube and a series of side arms to which the felicitous application of the finger would produce smoking at a rate that simulated the human. In fact, with an additional side arm, we could blow smoke rings.

The measurements that we made, although by no means as sophisticated as those of Resnik et al., indicated no changes in major cardiovascular parameters, oxygen consumption, or gas transfer in the fetus.

Dr. Resnik (Closing). First in response to Dr. Crenshaw, I believe your points are well taken, and I think it might be worthwhile to go back to the genesis of these studies in order to defend our decision to restudy this issue. When we began these studies, it was clear to us that the isolated effect of nicotine had not been investigated. Cigarette smoke had been treated as an entity and the various components had not been studied individually. Our initial studies demonstrated that nicotine has the capability, in this particular species, to decrease uterine blood flow, that it is mediated by catecholamines, and that the response could be blocked by pretreatment with an α-blocker.

In our subsequent studies we demonstrated that a dose of one-half milligram per minute produced nicotine concentrations that were four to six times higher than those observed in smoking humans but had no physiologic effects either on the ewe or the fetus.

However, both the earlier studies utilized single daily exposures to nicotine and our concern was that they did not simulate the human smoking event, in which there occurs repetitive exposure throughout the day. That was the reason why we "taxed" our resources, if you will.

With respect to your other questions, I am not aware of any chronic studies involving exposure to nicotine over several weeks or months.

In terms of turnover rates of catecholamines in the fetus, to my knowledge, at least with respect to nicotine exposure, this experiment has not been done.

We did study the effects of nicotine on uterine blood flow earlier in gestation. The same response is noted again at much higher doses.

Dr. Greiss mentioned that the combined effects of the various components in tobacco smoke presumably influence suboptimal outcomes of neonates. I agree with him entirely. We were interested in trying to isolate the physiologic effects of nicotine per se.

Can preterm deliveries be prevented?

Denise M. Main, M.D., Steven G. Gabbe, M.D., Douglas Richardson, M.D., and Sharon Strong, R.N., M.S.N.

Philadelphia, Pennsylvania

Our hospital serves poor, inner-city women who have a 17% preterm delivery rate. Middle-class women in San Francisco at high risk for preterm delivery have benefited from an antepartum program which emphasized patient education and close follow-up. Using a controlled, randomized design, we are investigating the impact of similar interventions. Patients determined to be at high risk before 18 weeks' gestation on the basis of the Creasy system are randomly assigned to the Preterm Labor Prevention Clinic or serve as high-risk controls. Sixty-four women assigned to the Preterm Labor Prevention Clinic and 68 high-risk control women have been delivered of their infants. No significant differences were noted for the percentages of preterm infants, mean gestational age, or birth weight. Preterm rupture of the membranes accounted for 40% of preterm deliveries in all high-risk patients. Thirty percent of preterm births were indicated for maternal or fetal reasons. The remaining 30% represented failure of tocolytic therapy. (Am J Obstet Gynecol 1985;151:892-8.)

Key words: Preterm births, tocolytic therapy, preterm rupture of membranes

Indigent women in the United States continue to experience extremely high preterm delivery rates. Perinatal mortality resulting from preterm births in this population is the major contributor to this country's relatively poor performance with respect to international perinatal statistics.[1] To have significant national impact, interventions designed to reduce the incidence of prematurity must benefit poor, inner-city women.[2, 3] The Hospital of the University of Pennsylvania serves such a population. In 1982, 16.5% of pregnant women who registered for prenatal care at the obstetric clinic of the Hospital of the University of Pennsylvania prior to 18 weeks' gestation were delivered of preterm infants.

Several investigators have attempted to identify prospectively those women most likely to be delivered before 37 weeks' gestation. Interventions designed to prevent preterm births could then be selectively applied to these high-risk women. Creasy et al.[4] have modified Papiernik-Berkhauer's risk scoring system and applied it prospectively to women in New Zealand. An initial score of ≥10 points on this screening survey predicted a 30% chance of preterm delivery. Repeating the risk assessment at 24 to 28 weeks' gestation identified an additional group of women likely to be delivered before 37 weeks' gestation. This scoring system was then used to screen women at the University of California (San Francisco).[5] Those patients receiving ≥10 points were assigned to a special clinic designed to identify preterm labor at an early stage. Interventions in this clinic included weekly or biweekly pelvic examinations to detect asymptomatic cervical changes and education of both staff and patients about the subtle signs of preterm labor. High-risk patients were provided easy access to the medical staff around the clock. With these measures, the overall institutional preterm delivery rate fell from 6.7% to 2.4%. The control population in this study included retrospective data from the same institution as well as historical and concurrent controls from an affiliated hospital.

Material and methods

Using a randomized, controlled study design, we are investigating the impact of similar interventions applied prospectively to a population at great risk for preterm labor. To assess the characteristics of our population, a retrospective chart review was first performed which included all black patients who presented to the obstetric clinic of the Hospital of the University of Pennsylvania at or before 18 weeks' gestation and were delivered at the Hospital of the University of Pennsylvania in 1982. One nurse investigator (S. S.) assigned a risk score using the Creasy method to each of these patients based on the information available at the first clinic visit. This risk assessment system identified 29% of these patients to be at high risk for preterm labor. They accounted for 48% of all preterm births and 62% of all preterm births due to preterm labor or preterm rup-

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Table I. Overall outcome by risk group: Retrospective data

Risk score	*High risk* (≥ 10)	*Low risk* (<10)	*Significance*
No.	157	377	
Gestational age (mean ± SD) (wk)	37.4 ± 4.6	38.9 ± 2.9	$p < 0.001$
Birth weight (mean ± SD) (gm)	2729 ± 825	3060 ± 2.9	$p < 0.001$
Risk score (mean ± SD)	15.5 ± 6.7	6.4 ± 2.1	$p < 0.001$
Deliveries <37 wk	42 (26.8%)	45 (11.9%)	$p < 0.001$
Deliveries <37 wk for PTL or PROM	37 (23.6%)	23 (6.1%)	$p < 0.001$
Birth weight <2500 gm	48 (30.6%)	46 (12.2%)	$p < 0.001$
Deliveries <37 wk and birth weight <2500 gm	35 (22.3%)	24 (6.4%)	$p < 0.001$

PTL = Preterm labor; PROM = preterm rupture of the membranes.

ture of the membranes (Table I). The correlation coefficient between gestational age and risk score was -0.33 with a total variance explained (r^2) of 11%.

Our prospective study was started in April, 1983. All black patients presenting to the obstetric clinic of the Hospital of the University of Pennsylvania at or before 18 weeks' gestation were interviewed by the same nurse specialist and assigned a risk score according to the Creasy system. A cutoff of 18 weeks was selected to allow sufficient time for medical intervention and still include a large number of patients who registered after 12 weeks' gestation. Those women scoring ≥ 10 points were assigned, by means of a random numbers table, either to a group that was offered participation in the Preterm Labor Prevention Clinic or to a group that served as high-risk controls. High-risk control patients were followed up by the obstetric residents in the routine or high-risk clinic at the discretion of the chief residents. Neither the physicians nor the high-risk control patients were informed of their high-risk status. Additionally, those women with scores of ≤ 10 were followed up as low-risk controls in the same routine clinics staffed by obstetric residents.

Patients agreeing to participate in the Preterm Labor Prevention Clinic were seen on a weekly or biweekly basis by one of two investigators (D. M. M. or S. G. G.) who performed a pelvic examination to assess early changes in cervical dilatation, effacement, consistency, and position. When significant cervical change was detected, uterine activity was monitored with a tocodynamometer. Patient education regarding the subtle signs of preterm labor was taught by the nurse-specialist and these lessons were reinforced by the physicians. Patients were taught to palpate for mild contractions on a daily basis. A 24-hour telephone "hot-line" was provided to assure easy patient access to medical care. Cerclages were performed when the patient's past obstetric history indicated cervical incompetence or when cervical effacement and/or dilatation were documented early in the second trimester and the patient had a history suggestive of cervical incompetence. Prophylactic tocolytic therapy and progestin treatment were not used. Bed rest and prohibition of intercourse were not routinely recommended. Inpatient management of all women including the Preterm Labor Prevention Clinic group was directed by the residents and staff on service, not by the primary investigators in this study.

The best obstetric estimate of gestational age at delivery was determined by analysis of last menstrual period, estimations of early uterine size, gestational age at which fetal heart tones were heard by fetoscope, and ultrasonic dating when done. Pediatric gestational age was based on the modification of Dubowitz testing by Ballard et al.[6] Babies of high-risk controls and Preterm Labor Prevention Clinic patients were examined by one neonatologist (D. R.) who was blinded as to the mother's study group. These examinations were used to correct obstetric dating if results of the two methods differed by more than 3 weeks. Visits to the labor floor, hospital admissions, deliveries, and diagnoses in all study cases were recorded prospectively by the nurse-investigator.

Statistical analysis was performed with the Statistical Package for the Social Sciences, and the programs for χ^2, t tests, and analysis of variance were used as appropriate.

Results

During the first 16 months of the study, 380 patients have been delivered of their infants. Approximately one third of the patients were high risk (132 of 380) and were randomly distributed into the Preterm Labor Prevention Clinic and high-risk control populations (Table II). Women in these groups experienced nearly one half of the total preterm deliveries (30 of 62) and almost one half of the preterm deliveries due to preterm labor or preterm rupture of the membranes (22 of 47). The ability of a risk score ≥ 10 to predict preterm delivery was poor with a correlation coefficient between gestational age at delivery and risk score of -0.14 and a total variance explained (r^2) of 1.9%.

Significantly more women in the Preterm Labor Prevention Clinic visited the labor and delivery suite with complaints suggestive of preterm labor than did gravid women in the high-risk control group (37.5% versus

Table II. Overall outcome by risk group

				Significance	
	PLPC	*HRC*	*LRC*	*PLPC vs. HRC*	*(PLPC + HRC) vs. LRC*
No.	64	68	248		
Gestational age (mean ± SD) (wk)	37.3 ± 4.3	37.3 ± 4.6	38.4 ± 3.4	NS	p = 0.05
Birth weight (mean ± SD) (gm)	2830 ± 840	2837 ± 813	3023 ± 748	NS	p = 0.03
Deliveries <37 wk	16 (25.0%)	14 (20.6%)	32 (12.9%)	NS	p = 0.05
Deliveries <37 wk for PTL or PROM	11 (17.2%)	11 (16.2%)	25 (10.1%)	NS	NS
Birth weight <2500 gm	14 (21.9%)	13 (19.1%)	37 (14.9%)	NS	NS
Birth weight <2500 gm and <37 wk	12 (18.8%)	11 (16.2%)	24 (9.7%)	NS	p = 0.04
Deliveries <37 wk and <2500 gm for PTL and PROM	8 (12.5%)	8 (11.8%)	14 (5.6%)	NS	p = 0.04

PLPC = Preterm Labor Prevention Clinic; HRC = high-risk control; LRC = low-risk control.

Table III. Primary reason for early delivery by risk group

	PLPC	*HRC*	*LRC*	*Significance**
Delivery <37 wk	16	14	32	NS
PTL, tocolysis failed or contraindicated	3 (18.8%)	6 (42.9%)	10 (31.3%)	NS
PTL, mature lecithin/sphingomyelin ratio	0	1 (7.1%)	2 (6.3%)	NS
PROM	8 (50.0%)	4 (23.6%)	10 (31.3%)	NS
Hypertension	0	1 (7.1%)	3 (9.4%)	NS
Bleeding	2 (12.5%)	0	3 (9.4%)	NS
Fetal distress	1 (6.2%)	1 (7.1%)	2 (6.3%)	NS
Other	2† (12.5%)	1† (7.1%)	2†‡ (6.3%)	NS

*Significance of both comparisons: PLPC versus HRC and combined high-risk groups (PLPC + HRC) versus LRC.

†Intrauterine fetal death before 24 weeks.

‡Medical complication at 34 weeks.

19.1%, p = 0.03), indicating that these women had an increased awareness of the symptoms of preterm labor. There was a tendency for more women in the Preterm Labor Prevention Clinic to be diagnosed as having preterm labor, treated with tocolysis, and admitted to the hospital, though these differences did not reach significance. A cerclage was performed in 9.4% of Preterm Labor Prevention Clinic patients and in 2.9% of high-risk control patients. Preterm Labor Prevention Clinic women averaged 13.3 clinic visits as compared to 9.2 visits per high-risk control woman (p = 0.15). An average of 14.9 pelvic examinations were performed on each Preterm Labor Prevention Clinic participant before final labor and 4.6 on each high-risk control patient (p = 0.03).

Despite the intensive interventions received by women in the Preterm Labor Prevention Clinic, the incidence of preterm delivery was similar in both the Preterm Labor Prevention Clinic and the high-risk control groups. Deliveries prior to 37 weeks occurred in 25.0% of the Preterm Labor Prevention Clinic women as compared to 20.6% of the high-risk control patients. Of the Preterm Labor Prevention Clinic participants, 17.2% were delivered of preterm infants because of preterm labor or preterm rupture of the membranes, a rate almost identical to the 16.2% experienced by high-risk control women. Low-risk control patients also experienced a very high preterm delivery rate, 12.9% overall, with 25 of 32 cases related to preterm labor or preterm rupture of the membranes.

Preterm rupture of the membranes and/or failed tocolysis were the most common indications for preterm delivery in each group (Table III). There was no unusual skewing of gestational age at delivery among women who were delivered early based on group assignment. Similarly, the distribution of infant weights of those weighing <2500 gm at birth did not differ significantly by group assignment.

The two high-risk groups were comparable with respect to age, gravidity, parity, number of early and late abortions, and gestational age at first visit (Table IV). There was no significant difference in the number of women who had a prior premature infant in the past, but the total and mean numbers of prior preterm deliveries in the Preterm Labor Prevention Clinic group were significantly higher than in the high-risk control group. Six patients in the Preterm Labor Prevention Clinic group accounted for 45% of the total prior preterm deliveries for the group.

Because it is inappropriate to express means for nonparametric scores, we have presented risk scores in categorical groups of five points each. There was a tendency for more high-risk control women to have scores between 10 and 14, although this was not statistically

Table IV. Comparability of study groups

	PLPC	*HRC*	*LRC*	*Significance**
Age (mean ± SD) (yr)	23.3 ± 5.1	22.5 ± 6.1	23.5 ± 5.3	0.39
Gravidity (mean ± SD)	3.7 ± 1.8	3.4 ± 1.8	2.8 ± 1.8	0.43
Parity (mean ± SD)	1.3 ± 1.6	1.3 ± 1.2	1.2 ± 1.5	0.99
Abortions <14 wk (mean ± SD)	1.0 ± 1.5	0.9 ± 0.8	0.6 ± 0.8	0.35
Abortions >14 wk (mean ± SD)	0.7 ± 1.5	0.3 ± 0.4	0	0.06
Prior preterm delivery (mean ± SD)	0.7 ± 1.3	0.4 ± 0.6	0	0.03
No. of women with prior preterm delivery	29 (45%)	22 (32%)	0	0.18
Gestational age, first visit	12.7 ± 3.4	12.8 ± 3.3	12.1 ± 3.3	0.87

*PLPC versus HRC groups.

Table V. Comparability of risk scores in high risk groups

	PLPC		*HRC*	
Risk score	*n*	%	*n*	%
10-14	28	44	42	62
15-19	11	17	13	19
20-24	12	19	6	9
25-29	6	9	4	6
30+	7	11	3	4

p = 0.16 by 2 × 5 contingency table.

Table VI. Percentage of preterm deliveries by risk score category

	Preterm deliveries	
Risk score	*n*	%
<10	32/248	12.9
10-14	13/70	18.6
15-17	4/24	16.6
20-24	6/18	33.3
25-29	2/10	20.0
30+	5/10	50.0

Differences among categories >10 are not significant.

significant (Table V, p = 0.16). Furthermore, there was no difference in preterm delivery rates by risk score category for women with scores between 10 and 30 (Table VI).

The subgroup of patients with two or more prior preterm deliveries for any reason constituted a remarkably high-risk population that experienced a preterm delivery rate of 67%. The uneven allocation of these nine patients between the two experimental groups (six to the Preterm Labor Prevention Clinic group, three to the high-risk control group) accounted for much of the difference in risk score between the groups. When these nine patients were removed from the analysis, the results remained unchanged, with 13% of each high-risk group delivered of preterm infants because of preterm rupture of the membranes or preterm labor.

Comment

The most striking finding in this investigation was the absence of any demonstrable effect of our rather extensive intervention. For all three measures of outcome (mean gestational age, mean birth weight, and percentage of patients delivered prior to term), the Preterm Labor Prevention Clinic and high-risk control groups were virtually identical. This study remains ongoing. However, the absence of any current evidence of improvement in the intervention group suggests that increases in sample size will be unlikely to demonstrate a significant prolongation in gestation. To exclude the possibility of an improvement of >4 days in gestational age, we calculated that a final sample size of 200 is needed for each high-risk group.

Why were our intensive efforts unable to reduce the incidence of preterm deliveries in our population? Certainly patients attending the obstetric clinics at the Hospital of the University of Pennsylvania are at extremely high risk for preterm delivery. Our Preterm Labor Prevention Clinic patients came regularly for their clinic appointments and sought care on the labor and delivery floor when early signs of preterm labor were noted. They developed strong relationships with the special teaching nurse and the physicians staffing the Preterm Labor Prevention Clinic. Perhaps the multitude of factors which contribute to preterm delivery in this population cannot be overcome by more intensive observation and counseling during a single pregnancy. Berkowitz[7] and other investigators have documented that low socioeconomic status, low pregravid weight, inadequate weight gain during pregnancy, lack of leisure-time physical activities during the pregnancy, and a negative attitudinal expression toward the pregnancy will increase the risk of preterm delivery.[8] Such characteristics are common among black women seeking obstetric care at our hospital. It is entirely possible that conventional medical therapy is not as effective against these factors, even with early detection and timely initiation of treatment.

Preterm rupture of the membranes was the greatest single contributor to preterm delivery in the high-risk study groups. Thirty-five percent of all preterm deliveries and 40% of preterm births in the Preterm Labor Prevention Clinic and high-risk control patients were attributed to preterm rupture of the membranes. Other investigators have also found preterm rupture of the membranes to be responsible for approximately one third of all preterm births.[9] Preterm rupture of the membranes is a relative contraindication to inhibition of preterm labor. Thus, the Preterm Labor Prevention Clinic program, with its focus on early detection of preterm labor and rapid access to tocolysis, may have little to offer this group of patients. At least one recent report has suggested that frequent vaginal examinations to assess cervical dilatation and effacement might actually contribute to preterm rupture of the membranes at term.[10] However, in our study preterm rupture of the membranes was not significantly more common in patients who had frequent cervical assessments.

An additional 34% of all preterm deliveries were indicated for maternal or fetal reasons. This high rate (5.5%) of intentional preterm deliveries reflects the underlying high-risk population and is nearly as high as the total preterm delivery rate at the University of California (San Francisco). Insofar as the Preterm Labor Prevention Clinic program cannot prevent pregnancy-induced hypertension, abruptio placentae, placenta previa, intrauterine growth retardation, or fetal distress, the only benefit for this group might be easier access to medical care.

Finally, 30% of all preterm deliveries represented a failure of tocolysis. Of these patients, 47% were identified as high risk and could have received the special intervention. A substantial positive impact in this group would be necessary to produce a significant improvement in the overall performance of the study population.

Our retrospective and prospective data demonstrate that there is some predictive value in the risk scoring system (positive predictive value = 23%, negative predictive value = 88%). The outcomes of the high-risk and low-risk groups are different with a high degree of statistical significance. However, the clinical value of this information is questionable. Only 30 of 62 patients who experienced preterm deliveries were correctly classified as high risk, a sensitivity of 48%. This limited sensitivity makes it an inadequate tool for discriminating term from preterm delivery in our population.

Creasy et al.[4] assessed risk for preterm birth during the first trimester and repeated the risk assessment at 26 to 28 weeks. In our study, patients were assigned to risk groups only at their first clinic visit. More than one third were found to be at high risk on initial screening. Screening early in pregnancy enabled us to separate the Preterm Labor Prevention Clinic study group from other clinic patients and thereby avoid any spillover for our interventional care to the high-risk control and low-risk control populations. It was hoped that identification of patients early in pregnancy would provide adequate time for the Preterm Labor Prevention Clinic to improve outcome. With such a large population already at high risk, rescreening was thought to be of little benefit. In addition, rescreening could have overwhelmed the resources of the Preterm Labor Prevention Clinic and mixed the study groups.

The question "Can preterm deliveries be prevented?" remains unanswered. The present study suggests that etiologic factors which may play an important role in producing preterm birth in one population and interventions designed to counteract these factors may not be universally applicable. Further research must be directed toward determining the causes of preterm delivery. As Eastman[11] noted almost 40 years ago: "Only when the factors causing prematurity are clearly understood can any intelligent attempt at prevention be made."

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ROY M. PITKIN, Iowa City, Iowa. Preterm birth clearly constitutes the major problem in perinatal medicine. It remains so in spite of the remarkable improvements in outcome provided by modern neonatal intensive care techniques and the development and widespread usage of pharmacologic agents for short-term suppression of labor during the past decade or so. Thus additional means of addressing the problem are required.

Dr. Gabbe has reported an extremely important clinical investigation to us today. He has tested the hypothesis that a defined intervention in prenatal care will lower the rate of preterm birth. The population, consisting of indigent urban black women with a prematurity frequency twice or more the national average, is ideal for such study. He used an established scoring system to identify subjects at particular risk and verified the accuracy of this system in his population. Then he applied, randomly and prospectively, an intervention suggested previously to be effective. In other words, keeping in mind the axiom, "A difference, to be a difference, must make a difference," he tested whether antepartum care including frequent visits to a seasoned and interested clinician, an intensive patient educational program, and around-the-clock access to consultation afforded any better outcome than more standard or customary care. It did not, an unfortunate result from the standpoint of our hopes but one which tells us that we had best look elsewhere in our efforts to deal with this most pressing of obstetric problems. Moreover, any disappointment we might feel regarding the negative findings should in no way detract from our admiration for a well-conceived and well-conducted clinical investigation.

I have only two questions, both relatively minor: (1) Would analysis of nulliparous and parous women separately provide any further insight? This may be important since Creasy et al. (reference 4 of article) have shown that their scoring system is much more applicable to multigravid women than to primigravid women, presumably reflecting the key role of previous obstetric experience. (2) We are told that 9.4% of the Preterm Labor Prevention Clinic patients, who had weekly or biweekly cervical examinations, and 2.9% of the high-risk control patients underwent cerclage operations; what was the rate in the low-risk control group and what was the outcome of the cerclage procedures?

Dr. Richard H. Schwarz, Brooklyn, New York. Dr. Gabbe, in the statistics that you presented, although you passed over this point saying it was not significant, I noted that the incidence of premature rupture of the membranes in your study patients was 50%. The incidence in the high-risk control group was just 29%. Was that difference statistically significant, and do you have data on the number of examinations done in the high-risk control women?

Dr. Richard Paul, Los Angeles, California. I believe Dr. Gabbe stated that there was a problem with frequent failure of tocolysis in these patients.

I am curious to know the agent or agents used in these patients. Dr. Gabbe mentioned that premature rupture of the membranes was a relative contraindication to inhibition of preterm labor.

Was tocolysis used in patients with premature rupture of the membranes?

Dr. Robert C. Cefalo, Chapel Hill, North Carolina. The State of North Carolina has a statewide program involving both the public and private sectors evaluating the preterm birth prevention program based on the Creasy/Herron model. Part of the evaluation is to look at the risk scoring tool.

Fortunately, we have many physicians who are using the same risk scoring tool. This will give us an evaluation of whether the risk scoring tool for the particular population is appropriate.

My question to Dr. Gabbe is, have you looked at the risk scoring tool and weighted the high-risk factors in your high-risk group? You may find out that your tool and the score of 10 are not appropriate for your population.

Dr. Charles Flowers, Birmingham, Alabama. We have apparently reached our ability to reduce perinatal mortality among infants weighing about 2500 gm. Our ability to manage major obstetric complications and monitor the condition of the fetus in utero has made congenital anomalies the principal cause of these infant deaths.

What are some of the common threads that link these problems? They tend to occur most frequently among lower social groups. Sixty-five percent to 85% of these pregnancies are unplanned and frequently unwanted. There is thus little motivation for self-discipline and prenatal care. These patients are under tremendous stress. They frequently do not know where they will be living during the next 3 months. They live in crowded houses where their families occupy the space appropriate for one. Frequently family and social problems associated with poor housing, poor food, and poor people do not lead to tranquility. These stresses elevate catecholamines, which may reduce uterine blood flow. A reduction in decidual blood flow reduces the nutrition to the membranes. Stress also incites the production of norepinephrine which may stimulate uterine contractions, thus causing premature labor. Uterine contractions alter the lower uterine segment and reduce the integrity of the cervical mucus plug. Bacterial invasions through the cervix and poor nutrition to the membranes make their rupture likely.

We are spending millions of dollars on tocolytic agents. We must balance the cost of these agents against the reduction in the incidence of low–birth weight infants. We may be spending money to no avail. Prematurity prevention clinics may be effective if we counsel patients, assist them in reducing the stresses in their life, give them support, and teach them to recognize the earliest signs of labor. The early use of tocolytic agents in combination with an attack on social problems may be effective in reducing these major obstetric problems. The problems of prematurity are social. Let us make our primary attack here.

Dr. Charles Mahan, Gainesville, Florida. Obviously, if you have seen the poster session, Florida thinks some form of this program is going to work. But it is important to study it and find out what worked in San Francisco that can be applied at other places.

I have a couple of questions.

One of the elements in our studies and in the San Francisco studies that seemed to pay off the most was having the patient see the same health provider every time she came back for a prenatal visit.

It was not mentioned whether that was done in this study.

Also, quite a bit of time was spent with those high-risk patients in the San Francisco study, a minimum of 20 minutes at a routine return visit. What were comparable times in the Philadelphia study?

And, last, was vaginitis detected and aggressively treated in your study groups?

Dr. Robert K. Creasy, Houston, Texas. First of all, Dr. Gabbe referred to a premature labor prevention clinic. I have never done anything to try to prevent premature labor in the past. We have been trying to prevent premature birth.

I was wondering if you could tell us, Dr. Gabbe, what you were doing to prevent the onset of premature labor rather than trying to stop premature labor.

Second, could you tell us what number of patients in each group were candidates for the inhibition of premature labor?

Dr. E. Stewart Taylor, Denver, Colorado. I am not surprised by Dr. Gabbe's conclusions: two thirds of prematurity comes from causes we do not understand or have methods to prevent. The incidence of prematurity is two to three times greater among the indigent than among private patients. Better prenatal care will not prevent two thirds of the cases of prematurity, particularly among the indigent population.

Through many years of studying this problem, I have concluded that low economic status and its associated problems of a lifetime of poor nutrition, poor hygiene, poor housing, and poor education are probably the causes for our high rate of prematurity in many areas of this country. These problems are social, not obstetric, but when they are solved, the prematurity rate will be reduced by one half to one third its present rate.

I do not think that the rate of prematurity can be reduced through better obstetrics.

Dr. Gabbe (Closing). In response to Dr. Pitkin's questions, the distribution of nulliparous and multiparous patients in the two study groups was the same. About a third of patients in the special clinic and in the high-risk control group were nulliparous and two third multiparous.

The incidence of preterm delivery was the same in both groups, and, in fact, the scoring system seemed to be most predictive in the high-risk nulliparous patients.

Cerclages were performed in six of the Preterm Labor Prevention Clinic patients and in two high-risk control patients. There were no cerclages in the low-risk control group. Of the six cerclages in the Preterm Labor Prevention Clinic patients, two women were delivered of term infants. The others were not, because of abruptio placentae and preterm rupture of the membranes. Of the high-risk control patients who had cerclages, one of the two did have a term infant.

I hope that answers your questions.

We were concerned as well, Dr. Schwarz, about the increased incidence of preterm rupture of the membranes in the Preterm Labor Prevention Clinic patients. It was not significantly higher, but it was a concern, just as we were interested to see that the incidence of preterm delivery for failed tocolysis seemed to be somewhat lower in the special clinic patients. Patients who came to the Preterm Labor Prevention Clinic made, on the average, 14 visits and had 14 pelvic examinations. The high-risk control patients made on the average nine visits and had between four and five pelvic examinations. So that is a concern.

As far as the agents used for tocolysis, we use ritodrine as our primary agent and then magnesium sulfate. Tocolytics are almost never given to patients with preterm rupture of the membranes.

We have looked, Dr. Cefalo, at the risk scoring system and we are now evaluating what factors might be more predictive.

We have also attempted to develop our own tool, looking at some of the factors that Dr. Flowers mentioned. I certainly agree with him.

Dr. Hobel has emphasized for some time the concern about stress, so, to try to get a better handle on this, we are looking at factors such as: Do you have a stable home situation? Have you had an argument with your boyfriend? And other general health factors: When was the last time you saw a doctor? When was the last time your children saw a doctor?

Dr. Mahan asked about the way the clinic was run. There were two health care providers who saw the patients at every visit, Dr. Denise Main or myself, so they were really only dealing with two staff physicians. We spent quite a bit of time with them and had our special nurse conduct a group therapy session, if you will, so that the patients were counseled and seen for at least 30 minutes on each visit.

I agree, Dr. Creasy, that perhaps the clinic should not have been called a Preterm Labor Prevention Clinic, but the Preterm Birth Prevention Clinic.

It was more common for patients in the special clinic to be seen on the labor floor, to be diagnosed as being in preterm labor, and to be hospitalized for it, but outside of the number of visits that they made to the labor floor, those treatment differences were not significant. Overall, 24 patients in the Preterm Labor Prevention Clinic groups visited the labor floor for preterm labor, 16 were hospitalized, and six received intravenous tocolytics. Of high-risk control patients, 13 came to the labor floor for preterm labor, seven were hospitalized, and only one received intravenous tocolytics.

Finally, I agree with Dr. Taylor who concurs with Dr. Flowers. Dr. Stubblefield said some time back that if we want our rate of preterm birth to be the same as Sweden's, perhaps we will have to have a society that is more like Sweden's.

Endometrial cancer: How does cigarette smoking influence the risk of women under age 55 years having this tumor?

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This analysis of the Cancer and Steroid Hormone Study, a multicenter, population-based case control investigation of hormone use by women of reproductive age and endometrial, breast, and ovarian cancer shows that cigarette smoking is not associated with either an increased or a decreased risk of endometrial cancer. This study included 437 women with endometrial cancer and 3200 control subjects, all of whom were between the ages of 20 and 54 years at the time of interview. The absence of any alteration of the risk of endometrial cancer and smoking was found consistently no matter which variable was used as a measure of smoking—ever or never smoked cigarettes, former or current smoking, light or heavy smoking, or age smoking began. (AM J OBSTET GYNECOL 1985;151:899-905.)

Key words: Endometrial cancer, smoking, tumor

The best estimates available show that each year nearly 40,000 women in the United States develop newly diagnosed cancer of the endometrium. Approximately 3000 women die annually from this disease. Risk factors for this tumor—obesity, nulliparity, exogenous estrogen use, hypertension, and diabetes mellitus—are well documented in standard textbooks and review articles; however, other factors that might influence the risk for women of acquiring this tumor, such as cigarette smoking or oral contraceptive use, have been studied only recently.[1, 2]

The role of cigarette smoking in relation to endometrial cancer is unclear. Three possibilities exist. First, cigarette smoking could increase the risk of women acquiring this malignancy. Women who smoke have a mortality ratio 20% to 30% higher than those who do not. In 1980 an estimated 48,100 women died of cancer that occurred at sites where smoking has an established carcinogenic effect; an estimated additional 71,300 women developed new malignancies of these same organs.[3] At least three of these organs—the kidney, pancreas, and urinary bladder—never come into direct contact with the particles in tobacco smoke. Recent evidence indicates that a fourth site—the uterine cervix—should be added to this list.[4] The next possibility is that cigarette smoking could decrease the risk of endometrial cancer. Such a reduced risk has been reported in humans. Moreover, because women who smoke experience natural menopause at a younger age[5] and because laboratory animals exposed to the constituents of cigarette smoke may undergo ovarian atrophy,[6] it is possible that smoking decreases the risk of endometrial cancer by reducing estrogen secretion. Finally, cigarette smoking might have no influence at all on the risk of endometrial cancer. This could occur if the organ-specific carcinogens found in cigarette smoke did not affect the cells of the endometrium. It could also occur if the carcinogenic effects of cigarette smoke on the endometrium balanced the ovotoxic, antiestrogenic effects.

To investigate the relationship of smoking to endometrial cancer in women aged 20 to 54 years, we analyzed data from the Cancer and Steroid Hormone Study, a multicenter, population-based case control investigation of hormone use and the risks of endometrial, breast, and ovarian cancer. We found that cigarette smoking does not increase a woman's risk of developing endometrial cancer.

Material and methods

The data in this report are from the completed 29-month Cancer and Steroid Hormone Study. This investigation is a multicenter, population-based case control study of the risk of endometrial, breast, and ovarian cancer. While the focus of this work is most specifically directed at hormonal factors, understanding the development of these neoplasms requires examination of the full range of known risks. Questions about cigarette smoking were therefore an important element in the interview of both case and control subjects.

The Cancer and Steroid Hormone Study is coordinated by the Division of Reproductive Health (formerly the Family Planning Evaluation Division) of the Center for Health Promotion and Education, Centers for Dis-

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From the Epidemiology Program Office and the Division of Reproductive Health, Center for Health Promotion and Education, Centers for Disease Control.

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Table I. Characteristics of women with endometrial cancer and controls

Characteristics	*Controls (%) (n = 3200)*	*Cases (%) (n = 437)*
Age (yr)		
20-29	7.8	1.1
30-39	26.1	11.4
40-44	16.8	12.4
45-49	22.9	22.2
50-54	26.4	52.9
Total	100.0	100.0
Race*		
White	85.8	91.1
Black	9.5	4.8
Other	4.7	4.1
Total	100.0	100.0
Body weight (lb)*		
≤125	37.5	26.2
126-145	36.9	27.4
146-165	14.7	14.2
>165	11.0	32.2
Total	100.0	100.0
Parity*		
0	11.5	26.2
1-2	33.7	36.6
3-4	38.5	29.2
>4	16.3	8.1
Total	100.0	100.0
Menopausal status*		
Premenopausal	31.4	24.5
Menopausal	68.6	75.5
Total	100.0	100.0
Menarche (yr)*		
<12	45.7	52.7
13-14	42.6	36.5
≥15	11.7	10.8
Total	100.0	100.0
Medical history*		
Infertility	4.4	6.4
Hypertension	22.7	38.8
Diabetes	2.9	6.9
Estrogen use	9.8	16.6
Fibroids	13.0	30.7
Oral contraception*		
Never use	46.9	57.8
Use <3 mo	5.6	9.2
Use ≥3 mo	47.4	33.0
Total	100.0	100.0
Alcohol use*† (gm/wk)		
None	16.4	20.4
<50	48.0	55.4
50-149	22.7	16.5
≥150	13.1	7.8
Total	100.0	100.0

*Percent distribution of controls standardized by the direct method to the age distribution of the cases.

†Because questions about alcoholic intake were not asked prior to August, 1981, no information on this topic is available on 31.6% of control or 23.6% of case subjects.

ease Control. Support and consultation for this work are provided by the Center for Population Research, National Institute for Child Health and Human Development, and by the National Cancer Institute. Enrollment of subjects was conducted by Surveillance, Epidemiology, and End Results centers of the National Cancer Institute in the following six areas of the United States: the metropolitan areas of Atlanta, Detroit, San Francisco, and Seattle; the States of Connecticut and Iowa. A detailed description of methods used in this study has been reported in an earlier article.[7]

Cases. Research subjects designated as cases were women aged 20 to 54 years who lived in one of the six reporting areas in the United States and were newly diagnosed between December 1, 1980, and December 31, 1982, to have histologically demonstrated primary endometrial cancer. These subjects were interviewed within 6 months after diagnosis; the average time from diagnosis to interview was approximately 3 months. The projected number of expected cases of endometrial cancer in the six areas nearly equalled the number actually ascertained during the study.

Of the 603 subjects who were initially interviewed, 437 (72%) met both the epidemiologic and pathologic case definitions for this analysis after careful review of interview information and histologic material. A panel of three pathologists agreed that all 437 subjects had an epithelial malignancy of the endometrium. Of those who were excluded, 99 were judged to have hyperplasia rather than malignancy and 19 were diagnosed as having carcinoma in situ. Of the remaining 48 women, 26 had undergone a gynecologic procedure (usually dilatation and curettage) with an unknown outcome which had been performed before the study began. This raised sufficient doubt as to whether their condition was newly diagnosed during the study period or whether it had been known before that time; thus these subjects were not included in the analysis. The remainder (22) had tumors that were either not endometrial or not epithelial in origin.

Controls. Research subjects who served as controls were women aged 20 to 54 years who lived within the same geographic area as the cases; they were selected by Waksberg's method of random-digit telephone dialing.[8] An appropriate proportion of potential controls in each 5-year age group was selected to match the age distribution for women with breast cancer enrolled in the study. Of those selected, 83% agreed to participate. Excluded were those women who before December, 1980, had had a dilatation and curettage with an unknown outcome or had had a hysterectomy prior to the time of interview. A total of 3200 women served as control subjects.

Interviews. A pretested, standard, 50-minute questionnaire was administered to each study participant in her home. The questionnaire focused on demographic factors, reproductive, contraceptive, and medical histories, and risk factors that included the use of exogenous estrogens and/or oral contraceptives. The inter-

Table II. Risk of endometrial cancer by status of cigarette use

Cigarette smoking status	*Cases**	*Controls†*	*Relative risk‡*	*95% confidence interval*
Never smoked	221	1,452	1.0	Referent
Ever smoked	215	1,743	0.9	0.7, 1.1
Former smoker	86	610	1.0	0.7, 1.4
Current smoker	129	1,133	0.8	0.7, 1.1

*Excludes one subject with cancer for whom smoking status was unknown.

†Excludes five control subjects for whom smoking status was unknown.

‡Relative risks are adjusted for age, body weight oral contraceptive use, alcohol use, menopausal status, hypertension, and estrogen use.

view was structured so as not to disclose the main hypotheses of the study. To elicit accurate histories of the use of hormones, we used the following two memory aids: (1) a book of photographs of all oral contraceptive preparations that have been marketed in the United States and (2) a calendar on which to record major life events around which hormone usage might be more accurately recalled.

Any subject who stated during her interview that she had smoked 100 or more cigarettes during her lifetime was classified as having "Ever Smoked." Those who had smoked fewer were treated as having "Never Smoked." Women grouped under the heading "Ever Smoked" were subdivided into current and former smokers. A former smoker was defined as a subject who had smoked 100 or more cigarettes but who had not smoked in the year prior to the interview.

Cumulative pack-years of cigarette smoking is a measure of the total exposure of an individual to cigarette tobacco smoke. A person who stated during her interview that she smoked an average of 20 cigarettes a day, i.e., one pack per day, every day for a year accumulated one pack-year of exposure. A person who smoked at this frequency for 5 years has accumulated five pack-years of exposure to cigarette tobacco smoke. Similarly, a person who smokes five packs or 100 cigarettes each day for a year has also accumulated five pack-years of exposure.

Analysis. Analysis of these data employed the method of Mantel and Haenszel[9] to compute crude and standardized odd ratios to estimate the relative risk estimates and Miettinen's test-based method[10] to compute confidence intervals. We used the Mantel extension of the Mantel-Haenszel procedure to test the statistical significance of linear trends.[11]

We used two methods to assess confounding and effect modification. First, using the Mantel-Haenszel procedure, we controlled the results in each table individually for potentially confounding variables including demographic factors, body weight, age at menopause, use of exogenous estrogens and oral contraceptives, and history of hypertension. We identified those variables that individually yielded a standardized relative risk estimate appreciably different from the unstandardized estimate. We then simultaneously controlled for these variables with use of the Mantel-Haenszel procedure. Second, we used logistic regression to assess the effects of the risk factors of interest while controlling for several variables considered a priori to potentially confound the results; among the variables included in the model were age, body weight, hypertension, use of exogenous estrogens, use of oral contraception, alcohol use, current smoking status, and cumulative pack-years smoked. Since the results from both the Mantel-Haenszel and the logistic regression procedures agreed closely, only the latter are presented. Finally, we followed the approach discussed by Schlesselman[12] with use of Woolf's test for heterogeneity to evaluate effect modification.

Results

Compared with control subjects, women with endometrial cancer were more likely to be older, of white race, weigh more than 165 pounds, be nulliparous, be postmenopausal, and have begun menstruating at a younger age. They were also more likely to give a history of infertility, exogenous estrogen use, hypertension, and diabetes mellitus (see Table I). These differences are all consistent with the known risk factors for endometrial cancer.

Table II shows that Never Smokers and Ever Smokers have the same risk of developing endometrial cancer. This is true whether one considers Former Smokers or Current Smokers. In subsequent analyses, therefore, no distinction will be made between former and current smokers; only the results for Ever Smokers will be presented.

Table III makes the most important point in this analysis. It shows that the level of exposure to cigarette tobacco smoke does not appreciably alter the risk of endometrial cancer for the women in this study. This statement is as true for those women with less than one pack-year as it is for those with 15 or more pack-years of smoking exposure. Likewise, these relative risk es-

Table III. Risk of endometrial cancer by cumulative pack-years of cigarettes smoked

Pack-years smoked	*Cases**	*Controls†*	*Relative risk‡*	*95% confidence interval*
None	221	1,452	1.0	Referent
<1	16	147	0.8	0.5, 1.5
1-4	31	286	0.9	0.6, 1.3
5-9	20	232	0.8	0.4, 1.2
10-14	17	204	0.8	0.5, 1.5
≥15	126	863	1.0	0.7, 1.2

*Excludes six subjects with cancer for whom number of pack-years smoked was unknown.

†Excludes 16 control subjects for whom number of pack-years smoked was unknown.

‡Relative risks are adjusted for age, body weight, oral contraceptive use, alcohol use, menopausal status, hypertension, and estrogen use.

Table IV. Risk of endometrial cancer by smoking status and selected characteristics

	Cases		*Controls*		*Relative risk**	*95% confidence interval*
	Smokers	*Nonsmokers*	*Smokers*	*Nonsmokers*		
Weight (lb)						
≤165	155	139	1,564	1,292	0.9	0.7, 1.1
>165	59	81	175	154	1.1	0.7, 1.7
History of high blood pressure						
Negative	136	131	1,396	1,191	1.0	0.7, 1.3
Positive	79	89	319	259	0.8	0.5, 1.1
Menopausal status						
Premenopausal	49	52	812	825	1.1	0.7, 1.7
Postmenopausal	156	155	856	588	0.8	0.6, 1.1
Estrogen use						
Never	160	166	524	1,288	1.0	0.7, 1.2
Ever	31	34	114	62	0.4	0.2, 0.9
Oral contraceptive use						
Never	112	138	585	561	0.8	0.6, 1.0
Ever	102	80	1,156	887	1.1	0.8, 1.6
Age						
<50	112	94	1,264	1,084	1.1	0.8, 1.6
50-54	103	127	482	363	0.7	0.5, 1.0
Alcohol use						
Never	22	46	103	195	1.0	0.5, 1.9
Ever	137	128	1,123	826	0.7	0.6, 1.0

*Relative risks are all adjusted by logistic regression for all factors given in this table except the one under study.

timates are not appreciably altered by taking into account the age at which the subjects began smoking.

Even though there does not seem to be an effect of smoking on risk of developing endometrial cancer, we searched meticulously to see if any subgroup of women might have their risk of this malignancy altered by cigarette smoking. Table IV shows the results of this search. None of the relative risks for these groups differed significantly from 1.0 except among the estrogen users. The other differences between the groups shown in Table IV and the referent population of nonsmokers could therefore have all resulted from chance. What is the likelihood that an association between smoking and the risk of endometrial cancer might exist but go undetected by chance alone? In a study with this many subjects, the 95% confidence intervals are so narrow that none has an upper limit greater than 2.1 or a lower limit below 0.3. The likelihood that a true association would be missed is therefore quite small. Nonetheless, while an increase in the risk of endometrial cancer in women who smoke is very unlikely, we cannot exclude a possible protective effect in some very specific, small group of women who smoke.

Comment

This analysis shows that cigarette smoking overall is not associated with appreciable alteration of the risk of endometrial cancer. This result is found consistently in this analysis no matter which variable is used as a measure of smoking—ever used or never used cigarettes, former or current use, light or heavy smoking (as measured by pack-years), or age smoking began.

This lack of association is unlikely to be artifactual. The study is population based, thereby minimizing the likelihood of selection bias. Moreover, the ascertainment of cases is high (92% of the expected number) and the response rates of the women in the study were also high, making bias very unlikely. All subjects were interviewed in a uniform manner and within a short period of time after diagnosis of disease. Uniform diagnostic procedures employing histologic specimens and requiring both initial microscopic diagnosis at the location of the Surveillance, Epidemiology, and End Results center and by a review panel of three carefully selected and highly qualified pathologists minimized information and classification bias. Confounding was controlled by means of two different analytic approaches, stratification and logistic regression analysis, both of which gave similar results.

The scientific literature on smoking and endometrial cancer is not clear. We found no reports showing an increased risk. Some studies found neither an increased nor a decreased risk. Others reported a protective effect that was not convincing (in one case the authors themselves were not persuaded). Von Lau et al.,[3] for example, published data in 1975 showing a relative risk of 0.6 for endometrial cancer among women who had ever smoked. Their findings, while statistically significant, did not show a dose-response gradient and included few enough women who had both cancer and an exposure to smoking so that confounding was difficult to exclude as an explanation of the apparent association. Williams and Horm[14] analyzed the Third National Cancer Survey in studying the role of tobacco and alcohol on cancer. They found a relative risk of 0.8; this result could have been due to chance or confounding by alcohol use or race. Weiss et al.[15] reported a relative risk of 0.4 for endometrial cancer among women who had ever smoked. These workers adjusted for the pertinent confounding factors and found no dose-response gradient. Even though this result was not likely to have been due to chance, the authors urged that it be interpreted cautiously because their study did not meet all the criteria for a causal association. Baron has analyzed work originally reported by two other groups of investigators. Both of these studies had data showing an odds ratio of less than 1.0 (0.7 and 0.8 respectively), but neither were statistically significant. The Walnut Creek study also reported odds ratios for endometrial cancer of less than 1.0 for women who smoked cigarettes.[16] This study dealt with 38 cases, whereas the other studies mentioned here each included more than 150 women with this tumor. In addition, Garfinkel[17] has analyzed endometrial cancer deaths; he, too, finds an odds ratio of approximately 1.0 (0.96). These studies are therefore consistent with the one reported by us in that none show cigarette smoking to increase the risk of women developing endometrial cancer. Our analysis does not support the finding of a protective effect, but we cannot exclude that possibility in some specific group of women, for example, those age 55 years or older, who were included in other studies than this one.

Both animal and human data suggest that this absence of risk might result from the influence that cigarette smoke or one of its components has on the ovary and therefore on estrogen secretion. Essenberg et al.[6] showed that mice had atrophic ovaries at autopsy after 2 to 3 months of exposure to cigarette smoke. At 6 to 8 months, damage was more severe and no graafian follicles were found in any of the ovaries. Rats injected with nicotine showed little change in ovarian morphology at 3 months, but there was a drastic decrease in follicles of all types after 6 to 8 months. Of further interest is the report of Gulyas and Mattison[18] that a single injection of 3-methylcholanthrene, a tumor-initiating polynuclear aromatic hydrocarbon found in tobacco tar, depletes the ovary of oocytes, thereby shortening the reproductive life span. The authors caution against extrapolating these findings to humans.

Furthermore, an antiestrogen effect of smoking is supported by human studies showing that menopause occurs at a younger age in women who smoke than in those who do not.[7] The control subjects in our study also had a younger age at natural menopause among women who smoked than among those who did not.

In closing, we point out that cigarette smoking is a major health hazard for women regardless of its effect on endometrial cancer. Overall mortality, chronic obstructive pulmonary disease, cancer of the lung, larynx, oral cavity, esophagus, and kidney, coronary heart disease, peptic ulcer, and impaired reproductive health are all much more serious problems for women who smoke than for those who do not.[3]

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This report was prepared by Carl W. Tyler, Jr., M.D., Linda A. Webster, M.S.P.H., Howard W. Ory, M.D., George L. Rubin, M.D.

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Discussion

Dr. Charles B. Hammond, Durham, North Carolina. The population-based, case-controlled study addresses the likelihood of an association of cigarette smoking with the risk of developing endometrial carcinoma. In general, the study is well designed and well executed with careful elimination of bias and confusing variables. The conclusions of the study are that cigarette smoking is not associated with either an increased or a decreased risk of endometrial cancer in women younger than 55 years of age. It is of interest that the absence of any alteration of the risk of endometrial cancer and smoking was found consistently, no matter which variable was used as a measure of smoking quantity.

I have several comments regarding this paper:

First, I believe that the use of multivariant statistical analysis was highly appropriate for this study. Had other, less rigorous statistical tools been utilized, there might well have been an effect reported, i.e., that cigarette smoking led to diminished average weight, which indeed was associated with diminished risk of carcinoma of the endometrium. The use of the appropriate statistical tool validates the study still further.

Second, 112 patients with unknown results of curettage were deleted from the group. I wonder if this might have introduced some small element of bias, although I doubt it.

Third, and most importantly, I am concerned about the selected age range of women evaluated. In the paper only women who were younger than 55 years of age were studied. The mean age of diagnosis of endometrial cancer in recent studies ranges between 50 and 60 years of age.[1, 2] Use of ages less than 55 years indicates that the authors have chosen to ignore more than 75% of cases of newly diagnosed endometrial cancer.[3] To exclude such a large cohort prohibits generalization of these data to women of all ages.

From the above-noted comments, I believe that emphasis should be made by the authors that these data apply only to women of ages less than 55 years and that the implications of these results cannot be applied at this time to older women. I believe the emphasis should be detailed in the discussion, abstract, and even in the title of the paper.

In summary, I believe that this work is important and provides further information regarding the epidemiology of diseases linked to cigarette smoking as well as epidemiologic data regarding the most common gynecologic malignancy, endometrial carcinoma.

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Dr. Howard Judd, Los Angeles, California. I just want to reemphasize a couple of the points made by Dr. Hammond.

I am fairly disturbed about the fact that the study was limited to people under 55 for a tumor that is basically a tumor of women over 55 years of age, and second, Dr. Tyler, I would ask you to comment. One of the major debates that has existed on the relationship of endometrial cancer and estrogen usage is the control group. Drs. Feinstein and Horowitz in New England have complained bitterly about the fact that control subjects were used that had not had endometrial sampling, and also, there is the point that Dr. Hammond made that your control group has had no endometrial sampling. I am not sure that it would influence your results or not, but I am concerned about purposely excluding that group.

Dr. Mortimer G. Rosen, Cleveland, Ohio. I have no difficulty with the study, and I would like my remarks not to be interpreted as a criticism of the methodology or the outcome of the study. However, in discussing the confounding variables I might add that in our work on cigarette smoking and alcohol we found multiple other associated behavioral variables such as drug intake which are also confounding variables.

My point, however, is that there is a tremendous amount of money that is being spent on the area of cigarette smoking in relationship to endometrial cancer. I am somewhat uncertain as to why someone would feel that cigarette smoking itself might be an important variable with a high relative risk for inducing endometrial cancer. I wonder if the author would explain that to me.

If one were looking at weight loss, for example, as one of the confounding or important variables here, then why not look into diet itself as a more important factor, and the availability of nutrition to the population rather than cigarette smoking itself as a means of lowering weight and altering risk.

Dr. J. Donald Woodruff, Baltimore, Maryland. I am concerned about the fact that we constantly comment on the fact that there are 39,000 to 40,000 new cases of endometrial cancer in the United States each year with approximately a 92% 5-year survival, i.e., 3200 to 3300 deaths.

This is unrealistic. I think we should recognize the fact that there is something wrong with the diagnosis of these cases, because everybody who treats endometrial cancer realizes that the average overall 5-year survival is approximately 65% to 70%. Thus it appears that there is a new diagnosis in approximately 10,000 cases or an error in reporting.

Dr. Tyler (Closing). One of the real advantages of presenting a paper to this society is that one's ideas can always get a sharp and insightful critique.

The concern that the discussants have about the age of the subjects reflects the fact that the principal focus of the Cancer and Steroid Hormone Study was on women of reproductive age. I had hoped that that had been made clear in the Methods section of the presentation, but I am certainly very receptive to Dr. Hammond's suggestion that it be given further emphasis; that is clearly where Dr. Judd also was leading me.

The issue of telephone use as a survey technique is one about which we are learning an increasing amount. At present in the United States the prevalence of telephones in households is well over 90%. The Surveillance, Epidemiology, and End Results center areas are selected in part so that telephone survey techniques can be used in identifying control subjects as they were in this study. Survey teams that do this work are not on the job exclusively from 9 to 5 and do not work a 5-day week. They make their phone calls on Saturdays and on Sundays and after conventional work hours so that women in the labor force are as likely as possible to be identified as control subjects. Moreover, a prospective control subject is not omitted from the study until there have been at least three phone calls made to each telephone number.

Dr. Judd asked about the issue of control subjects. The topic raised by Drs. Feinstein and Horowitz is one about which there is still a good deal of controversy. All I can say at this point is that the approach to identifying control subjects used in the Cancer and Steroid Hormone Study has had careful review not only by teams of epidemiologists and statisticians at the Centers for Disease Control but also by scientists both from the National Institutes of Health and universities.

Dr. Rosen raises an important issue about why cigarette smoking should be included as a possible risk factor. The Surgeon General reports that cigarette smoke contains in excess of 3600 different components. Among them are known and certifiable carcinogens such as arsenic and nickel. In addition, the metabolism of nicotine is being laid out in more detail and its relationship to nitrosamines, another group of chemical carcinogens, is becoming clear. I believe therefore that serious investigation of the role of cigarette smoking is warranted for every gynecologic cancer.

Dr. Hammond raised questions about the deletion of certain subjects and the most frequent cause for deletion was that these people had not undergone a full pathology panel review but had undergone a uterine sampling procedure prior to the time of the study, so that we were unable to determine whether they met the case definition or not in terms of their being an incident case, a new primary endometrial cancer, or not a case at all. When there was any doubt, we opted for the specificity of a precise diagnosis that we could demonstrate. That is why they were excluded.

The influence of oral contraceptives on the postmolar human chorionic gonadotropin regression curve

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Los Angeles, California, and Charleston, South Carolina

This article reports a retrospective analysis of 149 evaluable cases of molar pregnancy managed at Women's Hospital, Los Angeles County/University of Southern California Medical Center, from January 1977, through June, 1983. In the 84 cases prior to 1981, the patients received estrogen-progestogen oral contraceptives after evacuation while the 55 patients seen after that used nonhormonal contraceptives. The frequency of abnormal regression of the serum β-subunit of human chorionic gonadotropin in the two groups was not significantly different (22.6% in the hormonal contraception group versus 34.5% in the nonhormonal contraception group). The groups were compared for known and potential risk factors and were nearly identical with respect to patient age, parity, maternal blood type, and race. There were also no significant differences with respect to uterine size, preevacuation β-subunit of human chorionic gonadotropin serum titer >100,000 mIU/ml, and frequency of theca-lutein cysts. Gestational age was significantly shorter and the frequency of cases with a preevacuation β-subunit of human chorionic gonadotropin serum titer >250,000 mIU/ml significantly higher in the nonhormonal contraception group, indicating that the nonhormonal contraception group had a higher risk for abnormal human chorionic gonadotropin regression than the hormonal contraception group. We conclude that this study provides no evidence that the use of estrogen-progestogen oral contraceptives prior to human chorionic gonadotropin remission increases the risk for invasive mole or choriocarcinoma following molar pregnancy. (AM J OBSTET GYNECOL 1985;151:906-14.)

Key words: Contraceptives, molar pregnancy, trophoblastic disease

Stone et al.,[1] in 1976, and Stone and Bagshawe,[2] in 1979, reported from the United Kingdom that the use of estrogen/progestogen oral contraceptives before human chorionic gonadotropin (hCG) titer remission following molar pregnancy increased the risk for invasive mole and choriocarcinoma twofold to threefold. These reports have raised serious doubts about the safety of prescribing hormones for contraception during the early weeks after molar pregnancy and also during treatment for gestational trophoblastic neoplasia. Since it is a standard practice in the United States to prescribe hormonal contraceptives in this clinical situation (although Brewer et al.[3] recommended against it), it is important for other clinics to review their experience. Following the articles of Stone et al. two additional studies have been reported, one by Berkowitz et al.,[4] which reported no adverse effects, and one by Ho Yuen and Burch,[5] which did report adverse effects but only for those women who received oral contraceptives containing >50 μg of estrogen. Prompted by these reports, in 1981, we discontinued prescribing oral contraceptives to our patients following molar pregnancy in favor of nonhormonal contraceptives to study the effect this might have on the hCG regression time and frequency of abnormal regression. In this paper, we report the results of our study.

Material and methods

The records of all patients with evacuation of a molar pregnancy at Women's Hospital, Los Angeles County/University of Southern California Medical Center, between January 1, 1977, and June 30, 1983, were reviewed. The diagnosis was based upon the presence of vascular, edematous villi with trophoblastic proliferation on microscopic examination of the products of conception. During the period January 1, 1977, through December 31, 1980, estrogen-progestogen oral contraceptives were prescribed within the first 2 weeks after evacuation on a routine basis. The patients from this period constitute the hormonal contraceptive group. Beginning in January, 1981, nonhormonal contraceptives (foam and condoms) were routinely pre-

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scribed. This practice was continued until June 30, 1983. The patients with molar pregnancy at Women's Hospital during this time interval constitute the nonhormonal contraceptive study group.

Clinical data collected prior to evacuation included the patient's age, race or ethnic group, ABO blood type, gravidity, gestational age (weeks since the last menstrual period), gestational size, and state of the pregnancy, that is, whether it was intact or partly aborted. Laboratory evaluation consisted of chest x-ray film, quantitative analysis of the serum level of the β-subunit of hCG (β-hCG) by radioimmunoassay, blood cell counts, serum electrolytes, renal and liver function tests, and a thyroid profile. If the gestation was >16 weeks' size, arterial blood gases were also measured. The diagnosis was usually established by amniography or ultrasound. Evacuation was achieved by suction curettage followed by sharp curettage unless the products of conception had been largely aborted, in which case sharp curettage only was done. An occasional patient underwent evacuation with the use of intravaginal prostaglandins based on a clinical diagnosis of a missed abortion. Primary hysterectomy was carried out in those patients who were clinically stable and were no longer interested in childbearing.

During the period of this study, the antibody used in the β-hCG serum assay was changed four times with a concurrent change in sensitivity. Since the least sensitive of the assays had a normal hCG value of 5 mIU/ml, this value was used to determine the time to titer remission for all patients in the study.

Postevacuation follow-up of the patients with molar pregnancy consisted of weekly serum hCG measurement until titer remission was achieved. Titers were then measured monthly for 6 months if hCG regression was normal and for 12 months if hCG regression was abnormal. For the purposes of this study, abnormal regression was defined in terms of a rise or a plateau. A rise was present when the hCG titer exhibited at least a doubling of its numerical value on the basis of at least three measurements during a period of not less than 2 weeks. A plateau consisted of a series of at least four titers during a minimum of 3 weeks which demonstrated less than a doubling of the hCG titer with no net numerical decrease in its value over the time span of the plateau. All other hCG regression behavior was considered to be normal. Treatment was recommended for all patients whose titers showed a rise or plateau above 500 mIU/ml. Below the 500 mIU level, a longer period of observation was the rule.

In addition to weekly hCG titers, patients were questioned especially with regard to their compliance with contraception and the occurrence of uterine bleeding during follow-up. A pelvic examination was done at 2- to 4-week intervals to identify theca-lutein cysts and to evaluate uterine involution. Chest x-ray films and other tests were performed in follow-up only if the hCG regression was abnormal or in response to symptoms or findings suspicious for metastases or other significant disease.

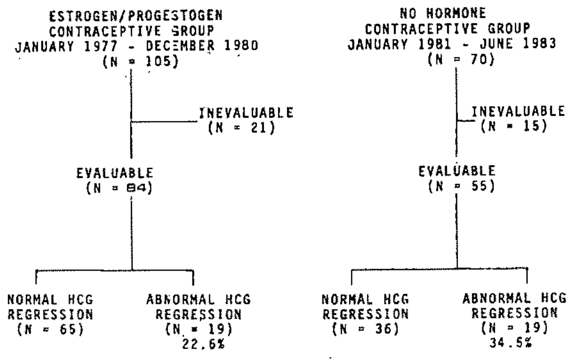
ESTROGEN/PROGESTOGEN CONTRACEPTIVE GROUP JANUARY 1977 - DECEMBER 1980 (N = 105)
INEVALUABLE (N = 21)
EVALUABLE (N = 84)
NORMAL HCG REGRESSION (N = 65)
ABNORMAL HCG REGRESSION (N = 19) 22.6%
NO HORMONE CONTRACEPTIVE GROUP JANUARY 1981 - JUNE 1983 (N = 70)
INEVALUABLE (N = 15)
EVALUABLE (N = 55)
NORMAL HCG REGRESSION (N = 36)
ABNORMAL HCG REGRESSION (N = 19) 34.5%

Fig. 1. Flow diagram of study, all molar pregnancies, January, 1977, to June, 1983 ($n = 175$).

Data were first analyzed by rankit or probit analysis to determine whether the distribution was normal or log-normal.[6] For sample sets with data approximating the normal distribution, the statistic Student *t* test was used to compare the populations. When the data set approximated a log-normal distribution, the nonparametric equivalent of the *t* test, the Mann-Whitney U test, was used. For large numbers, the appropriate Z transformation for the Mann-Whitney U statistic was performed.[7] For data sets where a yes-no answer could easily be discerned, an algorithm was developed and the results were calculated by χ^2 test with continuity correction when the cell contained less than seven, and, where appropriate ($n < 20$), Fisher's exact probability[7] test was used.

Results

A total of 175 women had molar gestations managed at Women's Hospital during the study period (Fig. 1). Of these, 36 were considered to be inevaluable (Table I). Among the 84 evaluable cases in the hormone study group, 24 (22.6%) exhibited an abnormal hCG regression curve while 19 (34.5%) of the 55 cases in the no hormone study group had abnormal hCG regression. These frequencies are not significantly different by χ^2 analysis ($p > 0.10$) although numerically there was a higher proportion of abnormal regression cases among the no hormone group.

A comparison of the demographic risk factors for the two study groups is presented in Table II. There were no significant differences with respect to maternal age, gravidity, parity, maternal ABO blood type, or race. In Table III are data comparing the clinical fea-

Table I. Reasons for inevaluability of patients in study

Reason	*Estrogen-progestogen contraceptive group*	*No hormone contraceptive group*
Received no hormones	4	–
Received progestogen only	4	0
Received estrogen-progestogen contraceptives	–	3
Treated by hysterectomy	3	2
Lost to follow-up too early	10	10
Total inevaluable	21	15

Table II. Comparison of demographic features of the estrogen-progestogen contraceptive study group and the no hormone contraceptive study group

Demographic feature	*Estrogen-progestogen study group*	*No hormone study group*	*Statistical comparison*
Patient age (yr)			
Mean ± SEM	23.46 ± 0.72	23.74 ± 1.00	t = 0.221 (NS)
Range	14-50	15-56	
>35	10 (11.9%)	4 (7.3%)	p > 0.95 (NS)
Gravidity			
Mean ± SEM	2.65 ± 0.11	2.50 ± 0.28	t = 0.441 (NS)
Range	1-9	1-11	
Primigravid	26 (30.9%)	23 (41.8%)	p > 0.95 (NS)
Parity			
Mean ± SEM	1.25 ± 0.16	1.18 ± 0.25	t = 0.148 (NS)
Range	0-7	0-10	
Maternal blood type			
A	19 (28.4%)	3 (25.8%)	p > 0.80 (NS)*
O	43 (64.2%)	20 (64.5%)	
B	4 (6.0%)	3 (4.0%)	
AB	1 (1.5%)	0	
Not known	17	26	
Race			
Hispanic	77 (92.8%)	47 (92.2%)	p > 0.90 (NS)†
White	2 (2.4%)	3 (5.9%)	
Black	2 (2.4%)	1 (2.0%)	
Oriental	3 (3.6%)	0	
Not recorded	0	4	

*A versus O.

†Hispanic versus white.

tures of the women in each study group. There was no significant difference in the mean gestational size of the molar pregnancies in the two study groups, the number of patients with a uterus >16 weeks' gestational size, the number that had a large-for-dates uterus, the number with a preevacuation hCG titer >100,000 mIU/ml, the proportion of cases with abnormal hCG regression, or the frequency of theca-lutein cysts. The sample populations were statistically different, however, in three respects. The no hormone group had on the average gestations of shorter duration, a larger proportion of cases with a preevacuation serum hCG titer >250,000 mIU/ml, and a longer time to spontaneous remission for patients with normal hCG regression (mean of 49.9 versus 59.3 days).

Further analysis of the hCG values was undertaken to determine whether any significant differences existed between the regression curves of the hormone and no hormone study groups.

The hCG values from the hormone and no hormone groups were segregated into abnormal and normal regressions. The values were examined at weekly intervals from week 3 to week 8. (The values for each week were obtained from the group of 3 days clustered around the specific week, for instance, week 3 = 21 ± 3 days). After week 8, there were too few data points per week to analyze. Each group was ranked and comparisons were made with the Mann-Whitney U test (Table IV). From week 3 to week 6, patients of both groups with abnormal regression curves were significantly different from the respective population that had normal regression, but there was no significant difference between the abnormal regression values of the hormone and no hormone groups nor was there

Table III. Comparison of clinical features of the estrogen-progestogen contraceptive study group and the no hormone study group.

	Estrogen-progestogen study group	*No hormone study group*	*Statistical comparison*
Gestational age (wk)			
Mean ± SEM	17.34 ± 0.65	15.56 ± 0.67	t = 1.833* (p < 0.05)
Range	6-34	5-32	
<15 wk	23/79 (35.4%)	28/51 (54.9)	p > 0.10 (NS)
Gestational size (wk)			
Mean ± SEM	16.56 ± 0.46	16.82 ± 0.52	t = 0.36 (NS)
Range	6-28	6-27	
>16 wk	40/81 (49.4%)	23/51 (45.1%)	p > 0.50 (NS)
Large for dates (>2 wk)	27/80 (33.8%)	24/48 (50.0%)	0.05 < p <0.10 (NS)
Preevacuation serum hCG titer			
>100,000 mIU/ml	46/70 (65.7%)	30/45 (66.7%)	p > 0.90 (NS)
>250,000 mIU/ml	20/70 (28.6%)	26/45 (57.8%)	p < 0.01*
hCG regression			
Normal	65	36	
Abnormal	19 (22.6%)	19 (34.5%)	p > 0.10 (NS)
Time to hCG remission			
<100mIU/ml			
Mean ± SEM	20.28 ± 1.04	27.95 ± 1.88	t = 5.514*
Range	3-39	8-51	
<5 mIU/ml			
Mean ± SEM	49.9 ± 2.40	59.35 ± 2.62	t = 2.508*
Range	10-101	23-87	
Theca-lutein cysts (>5 cm)	10/80 (12.5%)	13/52 (25.0%)	0.05 < p < 0.10 (NS)

*Statistically significant difference.

Table IV. Mann-Whitney U test comparison of normal and abnormal serum hCG regression values from estrogen/progestogen contraceptive study group and no hormone contraceptive study group

Week after evacuation	*Abnormal regression Hormone vs. no hormone*	*Abnormal vs. normal regression:*	
		Hormone	*No hormone*
3	NS*	p < 0.001	p < 0.001
4	NS	p < 0.001	p < 0.001
5	NS	p < 0.001	p < 0.001
6	NS	p < 0.001	p < 0.001
7	NS	NS	NS
8	NS	p < 0.001	p < 0.001

*Not statistically significant (p > 0.05).

any significant difference between normal regression values of the two groups. A scattergram of the abnormal regression values is presented in Fig. 2 as visual evidence of the high degree of congruence between the two groups.

Since the time to remission for normal regression is dependent upon the initial hCG titer, the preevacuation hCG values for the two groups were compared (Table V). The no hormone group, which had the longer average time to remission, also had a greater proportion of cases with an initial hCG titer >250,000 mIU/ml (57.8% versus 28.6%, $p < 0.01$). The occurrence of abnormal regression within each titer subgroup, however, was not significantly different between the hormone and no hormone populations. Because the mean gestational age of the two groups was also significantly different, the frequency of abnormal hCG regression was determined at various gestational ages for each study population (Table VI). There was no significant difference at any gestational age. However, abnormal hCG regression was significantly correlated with gestational age (<14 weeks versus 15 to 24 weeks, $p < 0.02$; 15 to 24 weeks versus >24 weeks, NS).

Comment

This study provides no evidence that estrogen/progestogen combination oral contraceptive use following molar gestation alters the natural history of this disease. Statistical comparison of a study population that used hormonal contraception with a study population that

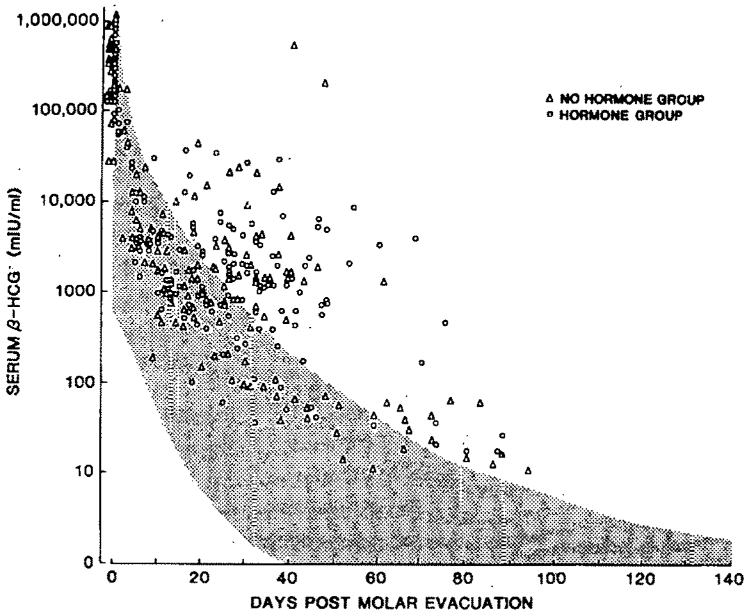

Fig. 2. Plot of serial hCG values from all evaluable patients in both study groups having abnormal regression. Values obtained after the plateau or rise criteria were fulfilled are excluded. The *shaded area* represents the range of values from both study groups having normal hCG regression.

Table V. Frequency of abnormal serum hCG regression related to preevacuation serum hCG titer

	hCG titer (mIU/ml × 10^3)						
Study group	*<25*	*25-50*	*50-75*	*75-100*	*100-250*	*250-500*	*>500*
Hormone	0/11	0/7	0/1	2/5	6/23	6/13	2/7
No hormone	0/4	1/4	0/6	1/1	2/4	6/16	6/10
Total	0/15	1/11 (9.1%)	0/7	3/6 (50.0%)	8/27 (29.6%)	12/29 (41.4%)	8/17 (47.1%)

did not demonstrated no significant difference with respect to the frequency of abnormal hCG regression or to the distribution of abnormal and normal hCG regression points as determined by the Mann-Whitney U test. The mean time to hCG titer remission (≤5 mIU/ml of serum) was significantly shorter for the hormone study group (49.9 versus 59.3 days). This difference is readily explained by the higher proportion of cases with an early gestation, a large-for-dates uterus, and a preevacuation hCG titer >250,000 mIU/ml (of 51 patients with an hCG titer >250,000 mIU/ml, 38 had a uterus that was >2 weeks large for dates). For two of these factors the differences were statistically significant. Since these are known risk factors for postmolar trophoblastic neoplasia, it would be expected that the no hormone group would have a larger proportion of cases with postmolar trophoblastic neoplasia as manifested by abnormal hCG regression. This was, in fact, the case although the differences were not statistically significant (hormone group, 19 of 65; no hormone group, 19 of 36; $0.10 < p < 0.20$). The study populations did not differ with respect to maternal age, gravidity, parity, ABO blood type, race, gestational age, and occurrence of theca-lutein cysts.

In this retrospective nonrandomized study carried out in a single hospital during two consecutive, relatively short time intervals and dealing only with patients with diagnosis, treatment, and follow-up at that hospital, it is not surprising that the two study groups were so similar in their demographic characteristics. There were significant dissimilarities with respect to the clinical features of the pregnancies with a bias favoring postmolar trophoblastic neoplasia in the hormone group. We interpret this to be a random bias occurring in two samples of a larger population which would be more accurately defined by combining the study groups. Thus it would be an error to infer that the differences in hCG regression suggest that combination oral contraceptives protect against the development of postmolar trophoblastic neoplasia, a notion which is exactly the opposite of that proposed by Stone and Bagshawe.[2] From their study of 611 women with molar

Table VI. Frequency of abnormal serum hCG regression related to gestational age of molar pregnancy based on menstrual dates.

	Gestational age					
	<15 wk		*15-24 wk*		*>24 wk*	
Study group	*n*	%	*n*	%	*n*	%
Hormone	11/29	37.9	8/41	19.5	0/9	0.0
No hormone	13/27	48.1	5/21	23.8	1/2	50.0
Total	24/56	42.8	13/62	21.0	1/11	9.1

pregnancy, they concluded that the use of oral contraceptives increased the risk for postmolar trophoblastic neoplasia by twofold to threefold (24.6% of 65 patients using oral contraceptives versus 8.8% of 490 patients not using oral contraceptives).

Why should the results of these studies be so divergent? To begin, the difference Stone and Bagshawe reported is not statistically significant ($\chi^2 = 3.39$, $0.05 < p < 0.10$) unless the 56 patients for whom the use of contraceptives was not recorded are included in the nonhormonal group. Furthermore, there appears to be a substantive bias in their study. In a group of young women for whom it was important to avoid pregnancy temporarily only 10.6% used oral contraceptives before hCG titer remission. How were the patients selected to receive oral contraceptives? No information is provided by the authors. The plausible explanations are: (1) that oral contraceptives were prescribed as a matter of practice 4 weeks or later after molar pregnancy termination, (2) that some of the women were given oral contraceptives specifically because the hCG titer remained elevated longer than usual, or (3) because vaginal bleeding occurred. All three of these factors are selective for trophoblastic neoplasia. The first explanation is supported by the observation that in 58% of Stone and Bagshawe's patients not receiving hormones, compared with only 7% of the hormone group, titer remission was achieved within the first month after evacuation. The proportion of patients with remission each month thereafter was reasonably similar for both groups. Our data show no such discrepancy in the spontaneous remission times of the two study populations. For the hormone group, the mean time to an hCG titer of <100 mIU/ml of serum (Stone and Bagshawe used <100 IU per 24-hour urine sample as the titer remission value) was 20.28 days (1.04 ± SEM) compared to 27.95 ± 1.88 days for the no hormone group ($p < 0.005$), a difference similar to that with 5.0 mIU/ml used as the point of remission. Furthermore, in our hormone group 83.3% of cases reached the 100 mIU/ml level during the first month (30 days) compared with 68.75% of the no hormone group. Even if the abnormal titer cases in our study are included, 65.5% of the hormone and 40.0% of the no hormone groups had hCG titers of 100 mIU/ml or less by the end of the first month after evacuation of molar pregnancy. Thus the differences in the interpretation or definition of abnormal hCG regression between this report and that of Stone and Bagshawe cannot account for the great disparity in the frequency of spontaneous remission during the first month after evacuation in the hormone and no hormone groups.

As the current and precedent studies[8-10] have demonstrated, the risk factors for postmolar trophoblastic neoplasia associated with molar gestation are numerous and patient populations nonhomogeneous. Furthermore, there may be geographically and/or racially determined biologic differences among women with molar pregnancy which vary from region to region and country to country. There are also obvious differences in diagnostic criteria of molar pregnancy (clinical versus microscopic; inclusion of transitional moles, hydropic abortuses, partial moles), postmolar trophoblastic neoplasia (what constitutes a rise in titer?), titer remission (<100 IU per 24-hour urine sample,[2] or <5 mIU,[11] <2 ng,[12] or <1.6 mIU[13] per milliliter of serum), and the type and quality of hCG assay. For these reasons, it is important to describe the study populations with respect to the existence of risk factors so that comparisons can be more fairly made. As the only study suggesting that the use of combination oral contraceptives after molar gestation is deleterious, the article of Stone and Bagshawe[2] compared their patient populations for maternal age alone. Berkowitz et al.,[4] however, compared their hormone and nonhormone study groups for maternal age, gravidity, histologic grade of molar tissue, pretreatment hCG titer (>100,000 mIU/ml), and whether they received prophylactic chemotherapy. The hormone group of 58 patients had a postmolar trophoblastic noeplasia rate of 18.9% (24 had received prophylactic chemotherapy) while the rate was 14.3% for the 42-patient barrier contraception group (16 had received prophylactic chemotherapy). The times to hCG remission for the patients with normal regression were 7.0 and 7.2 weeks, respectively, for the two groups. Thus they found no evidence that the risk for postmolar trophoblastic noeplasia was significantly influenced by the use of oral contraceptives in patients with similar risk factors.

Ho Yuen and Burch,[5] in 1983, reported data suggesting that the dose of the estrogen in oral contraceptives might be important in influencing the outcome of molar pregnancy. In their study three of 11 patients treated for postmolar trophoblastic neoplasia used oral contraceptives containing >50 μg of estrogen compared with four of 84 patients with molar pregnancy and spontaneous hCG remission ($p = 0.0317$ by Fisher's exact test). There was no difference, however, in the frequency of postmolar trophoblastic neoplasia for the total hormone group when compared to the no hormone group (11 of 108 versus 6 of 86, $p > 0.5$). These authors pointed out that oral contraceptives containing less than 50 μg of estrogen did not become available in the United Kingdom until approximately 1974. Since the patients in the study of Stone and Bagshawe were accessioned between September, 1972, and February, 1975, Ho Yuen and Burch concluded that the Stone and Bagshawe data were consistent with their own. Nearly all of our patients in the hormone study group were prescribed oral contraceptives containing 50 μg of mestranol plus 1 mg of norethindrone (Norinyl 1/50). Therefore, we cannot evaluate a dose-response effect in our study population. It is worthy of note, however, that Ho Yuen and Burch did not take into account the different estrogenic potency of mestranol and ethinyl estradiol. This resulted in the exclusion of 21 women from their high-estrogen group who used oral contraceptives containing 50 μg of ethinyl estradiol, approximately equivalent to 100 μg of mestranol.[14]

We have previously reported that there is no difference in the risk for postmolar trophoblastic neoplasia between patients receiving intramuscular medroxyprogesterone acetate (Depo-Provera) and those receiving combination oral contraceptives.[15] Based on our current analysis and a review of the relevant reports in the literature, we conclude that the use of estrogen-containing hormonal contraceptives immediately following molar gestation is a safe practice and that there is no acceptable clinical evidence that their use increases the occurrence of invasive mole or choriocarcinoma.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. HUGH R. K. BARBER, New York, New York. This is an important study which supplies a specific answer to the question about the influence of oral contraceptives on the postmolar hCG regression curves. The results have been subjected to critical statistical analyses.

Patients are and have been counseled on the importance of effective contraception for 1 year following passage of a molar pregnancy. Oral contraceptives are particularly useful in preventing pregnancy and are usually prescribed following passage of evacuation of a mole.

The current assays are very sensitive and provide a better monitoring system than existed in the past. Morrow et al. stated that during their study the antibody used in the β-hCG serum assay was changed four times. The least sensitive of the assays had a normal hCG value of 5 mIU/ml. This value was used to determine the times to titer remission for all patients in the study.

The articles by Stone et al., in 1976, and Stone and

Bagshawe, in 1979, caused concern over the use of oral contraceptives following a molar pregnancy. There was a reluctance on the part of the physician to give estrogen-progestogen oral contraceptives before hCG titer remission following molar pregnancy. This had medical as well as medical-legal implications. The work reported in 1979 commanded attention since it was presented by Bagshawe, a recognized world authority in the field of gestational trophoblastic neoplasms.

Morrow et al. have addressed this problem. They had two retrospective cohorts that they used for this study. The group of patients from January 1, 1977, through December 31, 1980, were given estrogen-progestogen oral contraceptives after a molar pregnancy. Following the article of Stone and Bagshawe, the use of oral contraceptives was discontinued by them and, starting in January, 1981, and continuing until June 30, 1983, non-hormonal contraceptives were prescribed.

The study included 84 available cases in the hormone group and 55 in the no hormone group. The criticism may be that since the numbers are so small it is difficult to give an accurate evaluation. Granted the series is small, but the patients have been well studied and the latest statistical methods have been used. This gives the conclusions a great measure of authority. It also provides a sound method for dealing with the sample populations that may have statistical differences.

Morrow et al. have studied the two groups by means of the Mann-Whitney U test, p values, Fisher's exact probability test, and rankit or probit analyses as well as several other statistical methods. It is obvious that their material has been well reviewed and their data subjected to critical analytical analyses.

The authors have provided a suggested answer for the difference between their findings and those of Stone and Bagshawe. The patients in Morrow's series were prescribed Norinyl 1/50, whereas Stone and Bagshawe provided higher doses of estrogen in their oral contraceptives. The second point of major importance is that Morrow et al. concluded that the use of oral contraceptives prior to hCG remission does notincrease the risk for postmolar trophoblastic neoplasia. The responsible physician can now prescribe the oral contraceptive without fear of increasing the risk of invasive mole and/or choriocarcinoma or the need to treat the patient longer with chemotherapy as reported by Stone and Bagshawe.

By using standard and up-to-date statistical analysis, Morrow et al. have provided a well-structured article presented in an orderly fashion. It also makes a contribution by clarifying a point of controversy in the literature. The references are excellent and current.

Dr. Roland A. Pattillo, Milwaukee, Wisconsin. Supportive in vitro data for Dr. Morrow's findings have recently been presented by Gall, McDonald, and the Buchsbaum group. These authors asked themselves the question, Do oral contraceptives modify hCG production in tissue culture? They evaluated the effects of ethinyl estradiol, norethisterone, and luteinizing hormone–releasing factor. They found absolutely no significant stimulation of hCG production in either group in tissue culture. Dr. Morrow's study is well designed and the data are very clear.

However, although the findings show no direct hCG stimulation, did the authors consider altered hCG forms? Were hCG α-subunit levels determined? Did they consider indirect modes of stimulation as have been reported for extramedian growth factors in breast cancer patients given estrogen?

Dr. Charles B. Hammond, Durham, North Carolina. I wonder if you could comment on the patients who did develop malignancy after molar pregnancy evacuation. Was there a difference in the categorization of the malignancy on the basis of histologic features, sites of metastasis, or other poor prognostic factors between the two groups in those who did develop malignancy?

Dr. Michael Newton, Chicago, Illinois. I have a couple of comments and a question.

First, when I went to Northwestern in 1977, I came under the strong influence of Dr. John Brewer. Therefore, I was convinced by Dr. Brewer that giving oral contraceptives to patients with hydatidiform mole was almost an evil deed. I did question this at the time, and we have thought about it since then. I wish we had done a prospective study, and in a way, Dr. Morrow's paper would have been better had it been prospective. But you cannot always do those things, and it is very useful information.

I would make a comment about the time it took the hormone levels to return to normal. About 45 to 55 days, I think, was given as the average, and this is considerably less than our average. Perhaps it relates to the sensitivity of the test used and the end point of 5 mIU/ml. We use 1.6 mIU/ml as an end point and it takes our titers longer to return to normal.

The question that I would ask him is related to Dr. Hammond's question. In a patient who has invasive mole or choriocarcinoma, does the giving of oral contraceptives increase the number of courses of chemotherapy or lengthen the time for the hCG level to return to normal? Is this an important factor when treating a patient with known disease?

Dr. John R. Marshall, Torrance, California. One of the benefits of oral contraceptives is the prevention of pregnancy. Oral contraceptives are recognized as being considerably more effective than barrier contraceptives.

Do you have any data on the number of women who became pregnant using the two contraceptive techniques? Additional pregnancies associated with the barrier methods would constitute an additional benefit of the oral contraceptives.

Dr. Samuel Solomon, Montreal, Quebec, Canada. Do I understand that you had five antibodies during the time of the 8-year period of study? Did the use of different antibodies cause some of the problems that you presented in the regression lines?

Did you get a chance to use a monoclonal antibody to β-hCG and compare the monoclonal to the wide-spectrum antibody in those patients where the regression line was more shallow—a slower regression line?

Dr. Morrow (Closing). There seems to be a discrepancy in the Stone-Bagshawe study which might account for the differences that we observed in relation to the risk of oral contraceptives.

Only 11% of their patients received oral contraceptives before titer remission, although approximately 55% of the women using contraceptives in the United Kingdom use oral contraceptives. The difference may be related to the time after evacuation the contraception was begun. Since 58% of Stone and Bagshawe's patients in the nonhormonal contraceptive group were in titer remission by 30 days (versus 7% for those in the hormonal contraceptive group) a large portion of the spontaneous remission cases would shift from the contraceptive to the noncontraceptive group if there was a 4-week delay before the contraceptives were started.

The influence of the estrogen dose, as I mentioned, could not be addressed in our study, but I would point out that the Ho Yuen study did not take into account differences in the estrogenic potency of ethinyl estradiol and mestranol. Consequently 21 patients who received 50 μg of ethinyl estradiol were excluded from their "greater than 50 μg estrogen" cases, while the seven patients included received 80 to 100 μg of mestranol.

In answering Dr. Pattillo's question about measuring the altered forms of hCG, our antibodies measured the β-subunit, free and combined. We did not measure the free α-subunit in these patients.

Dr. Hammond asked about the differences in the patients who had malignant complications in the two groups. There was one difference. There were more patients with metastasis (all to the lung) in the hormonal group (four of 19 versus zero of 19) but the difference was not statistically significant ($0.10 < p < 0.20$).

Dr. Newton raised the question about the time to remission in our patient population. It was shorter than that observed at Northwestern. I am sure it has entirely to do with our using 5 mIU/ml as the cutoff point for this study rather than 1.5 or 1 mIU/ml. Going from 5 to 1 mIU/ml may take another 2 or 3 weeks, in our experience.

Dr. Marshall asked about pregnancy prevention, and his point is an important one. However, the magnitude of the risk reported by Stone and Bagshawe, in our opinion, justified the use of barrier contraception until we were confident that oral contraceptives did not increase the cancer risk. We had very few pregnancies in either group. I am sure you realize that compliance is a problem, whether these patients are to use oral contraceptives or foam and condoms. However, there were more patients who got pregnant while using foam and condoms.

Dr. Solomon asked if we had ever used the monoclonal antibody for hCG. The answer is we have not.

Differences in twins: The importance of birth order

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Despite the clinical impression that firstborn twins do better than second-born twins, recent reports have shown no difference in perinatal mortality between them. In order to evaluate differences in twins, more sensitive means than perinatal deaths are necessary. This study examines differences between 80 firstborn and second-born twin pairs with respect to Apgar score, umbilical venous and arterial blood gas, and acid-base data. The umbilical venous and arterial blood Po_2, Pco_2, base deficit, pH, and lactic acid concentration were measured in paired samples and compared with the paired t test and χ^2 when applicable. Statistically significant differences favoring twin A, the firstborn, were found in 1-minute Apgar score, umbilical venous pH, Po_2, and Pco_2, and umbilical arterial Po_2. The other factors in umbilical venous and arterial blood did not show statistically significant differences. When these parameters were examined with respect to route of delivery, monochorionic and dichorionic twins, interval between twins, and vertex twins only, with the possible effects of malpresentation eliminated, the results persistently favored the firstborn twin. Thus it is unequivocally demonstrated that there are substantial differences at birth favoring the first twin, despite similar perinatal mortality for both. The data suggest that the second-born twin has potentially greater susceptibility to hypoxia and trauma. (Am J Obstet Gynecol 1985;151:915-21.)

Key words: Twins, umbilical blood gases, pH, lactate, birth order

There has been a long-standing clinical feeling that second twins are at a disadvantage.[1, 2] Recent studies have questioned this idea, finding no significant increased risk of neonatal death in the second-born twin in a series of 7001 live-born twins.[3] In another report on 305 twins, perinatal mortality also was not significantly increased for the second twin.[4] Nevertheless, breech presentation is higher[3] and the risk of large placental infarcts is greater in second twins.[4] Moreover, dizygous twins have obvious major differences such as sex, in addition to the difference in birth order. Therefore, there may be differences between firstborn and second-born twins which affect outcome other than mortality. This study seeks to uncover such differences.

Study design

A prospective study of all twin births at Bellevue and University Hospitals was initiated in 1979, resulting in the present study population of 80 unselected sets. There is a comparison of twin A, the firstborn, with twin B, the second-born, irrespective of their position in relation to the vagina before delivery. Thus, the firstborn twin by cesarean section might have been second-born in the case of vaginal delivery of that mother, by virtue of its higher position in the uterus. The twins were compared for weight and Apgar score at 1 and 5 minutes, as determined by a pediatrician not involved in the study. A comparison was made for umbilical venous and arterial blood parameters obtained after cord clamping with delivery of each twin. Blood was drawn with a 21-gauge needle and plastic syringe. The samples were analyzed immediately, or placed in vacuum tubes in an ice bath, and analyzed within 20 minutes of being obtained so that no significant effect of delay[5, 6] occurred. Blood samples were analyzed for pH, Po_2, Pco_2, and base deficit with the use of a Corning 165 analyzer and for lactic acid concentration with an enzymatic electrochemical method previously described by our laboratory.[7] The membranes were studied to classify twins as monochorionic and dichorionic, with the realization that monozygous twins can be dichorionic on occasion. Statistical analysis was carried out by the paired t test and χ^2 where applicable, with comparison of data for twin A and twin B.

Results

In a comparison of all twins for weight and Apgar score, the means showed no statistically significant difference in weight between twin A, 2474 ± 608 gm, and twin B, 2445 ± 615 gm. However, both the 1-minute and 5-minute Apgar scores were significantly higher for twin A. Moreover, the incidence of low 1-minute Apgar score was significantly greater for twin B, while the incidence of low Apgar score at 5 minutes was not

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Table I. All twins: Weight and Apgar scores

	Sets (no.)	*Twin A*	*Twin B*	*p*
Weight (gm)	78	2474 ± 608	2445 ± 615	NS
1 min Apgar score	80	8 ± 1.2	7.3 ± 1.9	0.001
5 min Apgar score	80	8.9 ± .8	8.6 ± 0.9	0.05
Incidence of Apgar score <7				
At 1 min	80	6.3%	17.5%	0.05
At 5 min	80	1.3%	3.8%	NS

Table II. All twins: Acid-base factors

	Sets (no.)	*Twin A*	*Twin B*	*p**
Umbilical venous pH	79	7.33 ± 0.06	7.31 ± 0.06	<0.01
Umbilical arterial pH	59	7.29 ± 0.06	7.28 ± 0.06	NS
Umbilical venous Po_2 (mm Hg)	74	32 ± 7	27 ± 9	<0.001
Umbilical arterial Po_2	53	19 ± 5	16 ± 5	<0.001
Umbilical venous Pco_2 (mm Hg)	72	38 ± 7	41 ± 8	<0.001
Umbilical arterial Pco_2 (mm Hg)	50	46 ± 9	48 ± 11	NS
Umbilical venous base deficit	72	5.6 ± 3.2	5.2 ± 3.2	NS
Umbilical arterial base deficit	50	4.6 ± 3.3	4.4 ± 3.7	NS
Umbilical venous lactate (mmol/L)	37	2.2 ± 1.02	2.28 ± 0.94	NS
Umbilical arterial lactate	29	2.39 ± 0.97	2.36 ± 0.84	NS

*Paired *t* test.

Table III. Twins delivered vaginally

	Sets (no.)	*Twin A*	*Twin B*	*p**
1 min Apgar score	24	8.2 ± 0.7	7.3 ± 2.2	<0.05
5 min Apgar score	24	8.9 ± 0.5	8.8 ± 0.9	NS
Umbilical venous pH	24	7.33 ± 0.05	7.30 ± 0.05	<0.05
Umbilical venous Po_2	24	30 ± 7	23 ± 6	<0.001
Umbilical arterial Po_2	17	20 ± 5	15 ± 7	<0.05
Umbilical venous Pco_2	24	36 ± 6	41 ± 7	<0.001
1 min Apgar score <7	24	0	12.5%	NS

*Paired *t* test.

(Table I). This suggests that there are differences present at birth, which may be influenced by medical intervention in the first 5 minutes of life.

Blood gas and pH analysis revealed no significant difference between the twins for umbilical arterial Pco_2, base deficit, lactate concentration, and pH. There was no significant difference in umbilical venous lactate or base deficit as well. However, there were statistically significant differences favoring twin A in umbilical venous pH, Po_2, and Pco_2 and in umbilical arterial Po_2 (Table II). Thus none of the factors studied favored twin B, while Apgar score, umbilical venous pH, Po_2, Pco_2, and umbilical arterial Po_2 favored twin A. What do these differences at birth, in favor of the first twin, actually mean? Further comparisons of vaginal and abdominal delivery, monochorionic and dichorionic twins, and the interval between firstborn and secondborn twins were carried out in order to elucidate the meaning of the observed differences in terms of their clinical implications.

The effect of route of delivery must be considered, since the second twin might suffer from more obstetric manipulation, inducing the observed differences. When twins delivered vaginally were compared, twin A was favored in 1-minute Apgar score, umbilical venous pH, Po_2, and Pco_2, and umbilical arterial Po_2. While the incidence of low 1-minute Apgar score in twin A was zero and in twin B, 12.5%, the numbers were too small for statistical significance (Table III). Comparison of twins delivered by cesarean section showed twin A favored by 1-minute and 5-minute Apgar score, umbilical venous Po_2 and Pco_2, and umbilical arterial Po_2. While both umbilical venous pH and incidence of low Apgar score were better for twin A, these numbers were not statistically significant (Table IV). Route of delivery was not the determinant of these

Table IV. Twins delivered abdominally

	Sets (no.)	*Twin A*	*Twin B*	*p**
1 min Apgar score	53	7.9 ± 1.4	7.3 ± 1.8	<0.02
5 min Apgar score	53	8.8 ± 0.8	8.5 ± 0.8	<0.05
Umbilical venous pH	52	7.33 ± 0.06	7.32 ± 0.06	NS
Umbilical venous Po_2	47	32 ± 8	28 ± 7	<0.01
Umbilical arterial Po_2	34	18 ± 5	16 ± 5	<0.02
Umbilical venous Pco_2	46	38 ± 8	40 ± 8	<0.05
1 min Apgar score <7	53	9.4%	20.8%	NS

*Paired *t* test.

Table V. Monochorionic twins

	Sets (no.)	*Twin A*	*Twin B*	*p**
1 min Apgar score	16	8.6 ± 0.6	7.9 ± 1.3	<0.01
5 min Apgar score	16	9.1 ± 0.3	8.8 ± 0.5	<0.02
Umbilical venous pH	16	7.32 ± 0.05	7.30 ± 0.06	NS
Umbilical venous Po_2	15	32 ± 6	27 ± 5	<0.02
Umbilical arterial Po_2	12	19 ± 5	18 ± 5	NS
Umbilical venous Pco_2	16	39 ± 4	42 ± 8	<0.05
1 min Apgar score <7	15	0	6.3%	NS

*Paired *t* test.

Table VI. Dichorionic twins

	Sets (no.)	*Twin A*	*Twin B*	*p*
1 min Apgar score	40	7.8 ± 1.3	6.9 ± 2.3	<0.01
5 min Apgar score	38	8.8 ± 0.7	8.5 ± 1.0	NS
Umbilical venous pH	39	7.33 ± 0.06	7.31 ± 0.06	<0.05
Umbilical venous Po_2	36	32 ± 8	26 ± 8	<0.001
Umbilical arterial Po_2	24	19 ± 5	14 ± 6	<0.01
Umbilical venous Pco_2	35	37 ± 7	41 ± 7	<0.01
1 min Apgar score <7	40	10%	27.5%	NS

differences, since they were present and in the same direction with vaginal and abdominal births.

Inherent differences might be responsible, particularly with respect to the zygosity of the twins. Analysis of the data in monochorionic twins showed significant differences favoring twin A in 1-minute and 5-minute Apgar score and umbilical venous Po_2 and Pco_2. Umbilical venous pH, umbilical arterial Po_2, and incidence of low 1-minute Apgar score were all better for twin A, but the small sample size did not allow for statistical significance (Table V). The dichorionic twins demonstrated the same differences favoring twin A in every parameter seen with monochorionic twins as well as in umbilical venous pH and umbilical arterial Po_2, except for 5-minute Apgar score (Table VI). The biochemical differences favoring the firstborn were seen in both dichorionic and monochorionic twins. Since the differences were clearly not related to zygosity, the effect of interval between the twins was evaluated. An interval of more than 5 minutes between twins revealed a statistically significant difference in favor of twin A in every determinant except umbilical arterial Pco_2 and pH, as well as incidence of low Apgar score. Even there, twin A had a zero incidence and the number of pairs was too small for statistical significance (Table VII). Furthermore, the twins delivered within 5 minutes of each other showed a statistically significant advantage for twin A as well, in Apgar score, umbilical artery Po_2, and umbilical venous Po_2 and Pco_2. Umbilical venous pH also was higher, but not significantly, in twin A (Table VIII).

The higher incidence of malpresentation in the second twin might explain the persistent disadvantage of the second-born twin at birth. Consideration of vertex-vertex pairs only, excluding all malpresentations of the second-born, permits evaluation of this possibility. Ta-

Table VII. Interval between twins of more than 5 minutes

	Sets (no.)	Twin A	Twin B	p
1 min Apgar score	18	8.4 ± 0.6	7.7 ± 0.9	<0.01
5 min Apgar score	18	9.0 ± 0.06	8.8 ± 0.4	NS
Umbilical venous pH	18	7.32 ± 0.06	7.27 ± 0.05	<0.01
Umbilical venous $\mathrm{Po_2}$	18	33 ± 7	23 ± 5	<0.001
Umbilical arterial $\mathrm{Po_2}$	11	22 ± 5	15 ± 4	<0.01
Umbilical venous $\mathrm{Pco_2}$	16	36 ± 5	43 ± 6	<0.001
1 min Apgar score <7	18	0	5.6%	NS

Table VIII. Interval between twins of 5 minutes or less

	Sets (no.)	Twin A	Twin B	p
1 min Apgar score	60	7.9 ± 1.3	7.2 ± 2.1	<0.01
5 min Apgar score	60	8.8 ± 0.8	8.5 ± 1.0	<0.05
Umbilical venous pH	59	7.34 ± 0.06	7.33 ± 0.06	NS
Umbilical venous $\mathrm{Po_2}$	54	31 ± 8	27 ± 7	<0.001
Umbilical arterial $\mathrm{Po_2}$	40	18 ± 5	16 ± 5	<0.05
Umbilical venous $\mathrm{Pco_2}$	54	38 ± 7	40 ± 8	<0.05
1 min Apgar score <7	60	8.3%	21.7%	<0.05

Table IX. Vertex twins

	Sets (no.)	Twin A	Twin B	p
1 min Apgar score	34	8.2 ± 1.3	7.3 ± 2.1	<0.01
5 min Apgar score	34	9.0 ± 0.6	8.6 ± 1.1	<0.02
Umbilical venous pH	34	7.32 ± 0.06	7.30 ± 0.07	NS
Umbilical venous $\mathrm{Po_2}$	32	31 ± 7	25 ± 7	<0.001
Umbilical arterial $\mathrm{Po_2}$	21	19 ± 4	15 ± 5	<0.01
Umbilical venous $\mathrm{Pco_2}$	32	37 ± 7	41 ± 8	<0.01
1 min Apgar score <7	34	5.9%	20.6%	NS

ble IX illustrates that the first twin does better in Apgar score at 1 and 5 minutes, umbilical venous $\mathrm{Po_2}$, $\mathrm{Pco_2}$, and umbilical arterial $\mathrm{Po_2}$. While umbilical venous pH and the incidence of low 1-minute Apgar score also favor twin A, these data are not statistically significant.

Comment

The increased perinatal mortality and morbidity in twin pregnancies[1-8] has made differences between firstborn and second-born twins particularly important. The present study demonstrates unequivocally that there are differences at birth between firstborn and second-born twins and that the first is favored. These differences are not due to route of delivery, interval between twins, presentation, or zygosity. Therefore, they do not appear to be iatrogenic or inherent in the individual twin. It seems more likely that the disadvantage to the second twin is actually in the order of birth and thus in some physiologic alteration following the birth of the first twin.

The most consistent findings are a higher umbilical venous $\mathrm{Po_2}$, a lower umbilical venous $\mathrm{Pco_2}$, and a better 1-minute Apgar score in twin A. Also better for twin A are umbilical venous pH, 5-minute Apgar score, incidence of low 1-minute Apgar score, and umbilical arterial $\mathrm{Po_2}$. This suggests that a phenomenon at the level of the placenta or the intervillous space is responsible. Differences in the umbilical venous blood reflect what is passing from the placenta to the fetus and influence umbilical arterial data and outcome. Thus reduced uterine size might decrease intervillous blood flow, resulting in less respiratory exchange between the second fetus, still in utero, and its placenta.[7] Additionally, placental function or blood volume might be affected by clamping the umbilical cord of the first twin, as has been shown in single pregnancies.[9]

Significant differences in blood gases, pH, and Apgar score between firstborn and second-born twins have been demonstrated. Their etiology does not appear to be specifically related to genetic or iatrogenic influences, to presentation, or to interval between twins. The second twin is at a biochemical disadvantage at the time of birth. These findings raise the issue of a possibly adverse effect of additional stress due to difficult delivery for the second twin. They may clarify the previous reports of poor outcome with assisted breech and version and extraction[10] and with low birth weight[11] for second twins. Such metabolic differences most likely indicate a reduced ability to compensate biochemically for hypoxic insult or a response to such an insult. Perhaps they confirm a biologic disadvantage for the second twin present from placentation on, possibly even determining birth order for most twins. This concept is difficult to accept but has been suggested by differences in cognitive test results with monozygotic twins who have dichorionic placentas.[12] Against this latter speculation is the absence of differences in lactate and inconsistent pH differences. Such a long-term difference should appear as a compensated metabolic acidosis, and this was not demonstrated for twin B.

With the use of sensitive biochemical indices, differences between firstborn and second-born twins favoring the firstborn are present, but their pathophysiologic features are not understood. The second twin clearly has less reserve to tolerate trauma or hypoxia at birth. Further studies are needed to elucidate the causes of the differences between twins and to recommend the optimal management of the second twin.

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Discussion

DR. WILLIAM N. SPELLACY, Chicago, Illinois. A search to determine the pathophysiologic effects of intrauterine crowding (with multiple fetuses) on pregnancy outcome may also shed light on control mechanisms for uterine blood flow, placental transfer, and fetal growth and development. Many clinical studies have demonstrated that the second fetus in twin pregnancies is at increased risk for malpresentation, prolapsed cord, and operative delivery with all of their potential morbidity and mortality.[1] The results of these studies have made obstetricians develop the management protocol for the second twin that all of us learned.

Today Dr. Young and his associates presented us with new data on the "second" twin. Their prospective studies of 80 twin sets with the use of both clinical and umbilical artery and vein blood gases suggests that the second-born twin is always disadvantaged in terms of blood oxygen, pH, and base deficit as well as Apgar scores. This seemed to be independent of abdominal versus vaginal delivery, time between delivery of infants, or zygosity. In this study, the "second" twin at cesarean delivery was not always the second presenting twin. While the trends were always constant, in some of the subsets the numbers were too small to achieve statistical significance.

The article suggests three questions which I would like to ask Dr. Young:

1. Individual twin results are not presented, but the closeness of some of the means suggests there were many second twins who were in a better oxygen state than the first twin. Did you analyze these data in terms of a different three subsets, namely, where the second twin was better than, equal to, and worse than the first twin, to see what pregnancy characteristics might be different and therefore causally related to impairing the second twin?

2. In the cesarean section cases some of the "second" twins were the first infant presenting. Did you analyze this group separately to see if the important variable was presentation order or birth order?

3. The important question is why and, in speculating on this, you suggest that increased uterine tone after contracting due to the removal of one infant could impair uterine blood flow enough to produce these results. Have you analyzed the status of the second twin in the cesarean section group where there were no traumatic births to look at time interval between infants and second twin status? Theoretically if oxygenation decreases after the first delivery then the longer the interval, the poorer the second infant will be.

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Dr. Raphael Jewelewicz, New York, New York. I have one comment and one question. If you have read the Bible you know that Rebekah was barren and Isaac prayed to the Lord who blessed her with twins. It is stated "the children struggled together within her." When they were born, twin A was Esau, "And after that came his brother out, and his hand took hold on Esau's heel" (Genesis 25).

Thus twin B, Jacob, was trying desperately to be first. Who knows, maybe if the sequence of birth had been different, our world today would be different.

The question relates to anesthesia. You have not mentioned anything about anesthesia, which may be of considerable importance. How many of these patients were under anesthesia and what type? What was the duration of anesthesia? Is there any difference in the various parameters that can be related to anesthesia?

Dr. John W. C. Johnson, Gainesville, Florida. Dr. Young, did you study the umbilical arterial pH? Would this not be a better reflection of how the second twin tolerated any theoretical disadvantage?

Dr. Richard Paul, Los Angeles, California. Could Dr. Young please tell us the incidence of cesarean section in this twin study group of patients? What was the occurrence of preterm delivery of the low–birth weight infants, and was there a disproportionate contribution to adverse outcome by the very small baby?

Dr. Frank Chervenak, New York, New York. How do these data relate to the very controversial issue, is breech delivery an acceptable way to manage the second twin in malpresentation?

Dr. Joe Leigh Simpson, Chicago, Illinois. Were there any anomalies in any of the 80 sets of twins? That is a relatively large set not to have had at least one. If so, how were they handled? Were these data eliminated or what?

Dr. Young (Closing). The questions that Dr. Spellacy asked confirmed that the thoughts I have been following up on are the same thoughts that the rest of you have.

Dr. Spellacy is quite correct in what he surmised about the closeness of some of the means. It was something that I was concerned about. Were these differences in terms of incidence of some significance? We looked at the distribution of the biochemical differences in terms of which twin had a better result. Was it better in twin A, was it better in twin B, or was it the same for both twins? When you break it down this way, and these are raw numbers, you still find that there is a statistically significant difference favoring twin A in Po_2 in the umbilical vein, Po_2 in the umbilical artery, Pco_2 in the umbilical vein, and Pco_2 in the umbilical artery.

In the second question, there was a concern about the birth order as opposed to the presentation order, and we deliberately excluded the presentation order in these cesarean section twins because we were focusing specifically on birth order. In the group of patients who had cesarean sections, the important thing was which baby was delivered first rather than which baby presented first. As Dr. Spellacy correctly stated, a very substantial number of the babies were delivered in a birth order which differed from their presentation order. We did not separate these as a subset, and as we continue this study and have large enough numbers, it might be interesting to look at that particular question. However, in all of the other circumstances, we did have a presentation order which coincided with birth order.

It reminds me of a comment somebody once made when we discussed these data. They suggested that the proper way to handle this problem was always to deliver twin B first.

With respect to Dr. Spellacy's third question, regarding time interval, where there was a cesarean section with a prolonged time interval between twins, we did this analysis. I do not have the data here with me, but there was a relatively poor regression analysis for long-term interval between first and second twin and deterioration. In other words, it appears that when you analyze the data for differences between twins in the range of 5 minutes or more, you can demonstrate a significant change, but as you extend the interval between twins further and further, you do not get a progressive deterioration. Perhaps the phenomenon is one which occurs rather rapidly and is not progressive, so other factors that we are concerned about with the second twin probably come into play when there are long intervals between twins.

Certainly, in the studies reported, for example, on vaginal delivery of the first twin followed by abdominal delivery of the second twin, the results are relatively poor for the second twin. This is usually because there are many other factors which come into play with a prolonged delay between twins. In the very interesting study by Evrard and Gold[1] on this, pointing out a one fourth perinatal mortality with second twins delivered this way, I think we have a clear demonstration that factors such as premature separation of the placenta, anesthesia, and various other concerns become important after the initial few minutes. The phenomenon is no longer a physiologic one at that point.

With respect to Dr. Jewelewicz' question, to which my last remarks lead us, the consideration of anesthesia is something that we studied. I did not have the opportunity to include all of the data that we have analyzed, but it does not make any difference whether the patients have epidural anesthesia or local or pudendal anesthesia, and we did not have enough patients delivered with general anesthesia to come to a specific conclusion on that. All of the differences between twins are consistently seen with the various kinds of anesthetics that we did use.

Dr. Johnson's question about umbilical artery pH was something that I mentioned, as we did look at the umbilical artery pH. It was not significantly different in almost every instance.

Dr. Paul asked about cesarean section. The frequency of cesarean section with twins was about a half to two thirds, particularly with the small twins, and this, I think, is the drift of his question. I have the data comparing premature and term twins. There was a significant difference in 1-minute Apgar score, umbilical venous pH, Po_2 in the umbilical vein and the umbilical artery, and Pco_2 in the umbilical vein. The term twins analyzed separately showed no significant difference in pH, significant difference in umbilical venous and umbilical arterial Po_2, umbilical venous Pco_2, and Apgar scores at 1 and 5 minutes. When these data were analyzed in terms of premature and term twins the differences were still consistent. Twin A was still ahead.

Dr. Chervenak's question about breech delivery with the second twin is one to which I cannot give an authoritative answer any more than all of you can at this particular moment. I would certainly suggest that gentle delivery, particularly for the small second twin who presents as a breech, is something that we must try our best to accomplish.

Finally, Dr. Simpson asked a question about anomalies. In this particular series we excluded anomalies before we started looking at the data.

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Influence of cytoplasmic steroid receptor content on prognosis of early stage endometrial carcinoma

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The clinicopathologic associations and effect on prognosis of cytoplasmic steroid receptor content were studied in 168 patients with clinical Stage I and II endometrial carcinoma. Cytoplasmic estrogen receptor status was associated ($p < 0.01$) with histologic differentiation, nuclear differentiation, and histologic documentation of extrauterine metastases. Progesterone receptor status was related ($p < 0.05$) to histologic differentiation and histologic cell type, and combined estrogen receptor/progesterone receptor status was associated ($p < 0.05$) with histologic differentiation, peritoneal cytology, extrauterine metastases, and histologic cell type among the 105 patients who had determination of both estrogen and progesterone receptors. Single-factor analysis revealed significant ($p < 0.05$) effects of estrogen receptor status, progesterone receptor status, and estrogen receptor/progesterone receptor status on disease-free survival. All other clinicopathologic features significantly ($p < 0.05$) affected prognosis, except for peritoneal cytology. With use of stepwise regression analysis of proportional hazards, estrogen receptor, progesterone receptor, and combined estrogen receptor/progesterone receptor status were significant independent prognostic factors, replacing histologic assessment of glandular or nuclear differentiation in the models. These data suggest that receptor status of primary endometrial carcinomas provides important information relevant to tumor behavior which complements the information provided by conventional clinicopathologic analysis. (AM J OBSTET GYNECOL 1985;151:922-32.)

Key words: Endometrial cancer, steroid receptor, prognosis

Cytoplasmic estrogen receptor– and progesterone receptor–binding proteins have been demonstrated to be necessary for steroid hormonal function in steroid target tissues and their malignancies. The presence of significant amounts of estrogen and progesterone receptors has been shown to be important in determining prognosis and response to therapy for breast carcinomas. Among gynecologic malignancies, the receptor content of endometrial carcinomas has been the most extensively studied. Documented associations of receptor content with histologic differentiation,[1-3] response to therapy,[1, 3-5] and possibly prognosis[1, 6] of endometrial carcinoma have been reported. Recently Martin et al.[6] reported that the presence of cytoplasmic estrogen receptors was a significant prognostic factor in determining survival following primary therapy of 87 patients with endometrial carcinoma of all stages.

The majority of women with endometrial carcinomas are diagnosed and treated at an early clinical stage. Many clinicopathologic factors have been demonstrated to be of value in predicting prognosis of early endometrial carcinoma. These factors include histologic grade, depth of myometrial invasion, cervical involvement, presence of extrauterine and lymph node metastases, and presence of malignant cells in peritoneal washings.[7-10] Histologic subtypes and nuclear differentiation may also be important in determining prognosis of early endometrial carcinoma.[7, 10]

Although cytoplasmic steroid receptor content of primary endometrial carcinoma may be an important prognostic factor, the data reported to date are inconclusive. This study evaluates estrogen and progesterone receptor status as independent prognostic factors for early endometrial carcinoma while controlling for known clinicopathologic prognostic factors.

Material and methods

Two hundred seventeen patients with clinical Stage I and II endometrial carcinoma treated at the Duke University Medical Center from 1976 through June, 1982, were available for cytoplasmic steroid receptor analysis. Tumor specimens were obtained at the time of initial evaluation and therapy. The presence of tumor and histologic pattern were histologically confirmed in the tissue specimen submitted for steroid receptor analyses. Thirty-eight patients were excluded

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because of insufficient tumor in the tissue submitted for receptor analyses, and two patients had progesterone receptor analysis only. Nine patients with clinical Stage I or II endometrial carcinomas had gross residual disease after primary therapy. The 168 patients with estrogen receptor and/or progesterone receptor data who were free of disease following primary therapy are the subject of this study. Treatment consisted of total abdominal hysterectomy and bilateral salpingo-oophorectomy in all patients. One hundred thirty patients underwent selective pelvic and para-aortic lymphadenectomies. Adjuvant radiation therapy, intraperitoneal chromic phosphate 32 instillation, and progestin therapy were utilized without regard to receptor status. Patients were followed until recurrence or death from intercurrent disease. Median follow-up was 25 months (range, 1 to 74 months).

Histology material. Pathology review was performed by two observers for categorization of tumors without knowledge of receptor content or clinical course. Permanent hematoxylin-eosin–stained sections from the tissue specimen submitted for receptor analyses were utilized to confirm malignancy. Histologic differentiation was categorized by International Federation of Gynecology and Obstetrics (FIGO) criteria[11] into well-differentiated, moderately differentiated, and poorly differentiated lesions. Nuclear differentiation was graded on a scale of 1 to 4.[7] Grade 1 nuclei were small and uniform in size with a regular chromatin pattern, whereas grade 4 nuclei were bizarre and pleomorphic, containing clumped chromatin and prominent, often multiple nucleoli. Grade 2 and 3 nuclei were intermediate in size and chromatin pattern.

Lesions were classified into adenosquamous carcinoma, adenoacanthoma, papillary adenocarcinoma, clear cell carcinoma, and adenocarcinoma. When squamous elements were identified, the lesions were classified as adenosquamous carcinoma or adenoacanthoma, depending on the presence of malignant squamous elements or benign squamous metaplasia according to the classification of Ng and Reagan.[10] When more than 50% of a lesion was composed of papillary or clear cell elements, it was categorized as papillary adenocarcinoma or clear cell carcinoma, respectively.[7] Lesions without other specific histologic features were designated as adenocarcinoma.

Additional surgical and histologic variables analyzed included extent of myometrial invasion, closest myometrial invasion to serosa, histologic documentation of extrauterine metastases, and peritoneal cytology. Cervical involvement of the hysterectomy specimen was characterized as none, invasion of endocervical glands only, or involvement of cervical stroma.

Steroid receptor analyses. Cytoplasmic estrogen and progesterone receptor binding was analyzed on fresh tissue or tissue fresh-frozen in liquid nitrogen by means of methods previously described.[1,2] Sucrose density gradient analysis and multiconcentration analysis with use of dextran-coated charcoal were performed for both estrogen and progesterone receptors when sufficient tissue was available. Cytoplasmic estrogen receptor was identified by inhibitable binding in the 8S component of sucrose density gradient analysis, whereas progesterone receptor was identified in the 6S component.[1] Identification of receptor by dextran-coated charcoal analysis required demonstration of inhibitable, high-affinity ($K_d < 10^{-9}$) cytoplasmic binding analyzed by the methods of Woosley and Muldoon[12] and Scatchard.[13] Frequently there was insufficient tissue for performance of all receptor assays. Previous reports have documented correlation of the two methods of receptor analysis in endometrial carcinomas.[1,2] Therefore precedence for assigning quantitative and qualitative receptor binding was given to the sucrose density gradient analysis value, with its greater specificity for functional receptor.[14] Cytoplasmic estrogen receptor content was determined for 168 patients, with 14 having only dextran-coated charcoal analysis. One hundred five patients also had progesterone receptor analysis, with only dextran-coated charcoal analysis used for 32. For the purposes of this study, bindings of >10 fmol/mg of protein by sucrose density gradient analysis and >15 fmol/mg of protein by dextran-coated charcoal analysis were considered to be positive.

Statistical analysis. Multiple clinical and histologic variables were analyzed for association with estrogen and progesterone receptor binding by means of χ^2, Fisher's exact test (two-tail), and Pearson's test for linear correlation where appropriate.[15] Survival estimates were determined by use of the Kaplan-Meier product limit method based on the most recent clinical status.[16] Deaths from intercurrent disease were considered censored observations according to clinical status of disease. The relative effect of clinicopathologic and receptor variables on survival were determined by means of Cox's proportional hazards regression model.[17]

Results

One hundred sixty-eight patients had analysis of estrogen receptors and 105 had analyses of both estrogen and progesterone receptors. The median age was 63 years with a range of 30 to 92; 86% were older than 50 years. The clinical stage was 1A for 102 (60.7%), 1B for 49 (29.2%), and II for 17 (10.1%) of the patients. Carcinomas were well differentiated in 67 (39.9%), moderately differentiated in 64 (38.1%), and poorly differentiated in 37 (22.0%) of lesions. Nuclear differentiation was similarly distributed, with 48 cases (28.5%) having grade 1, 63 (37.5%) grade 2, 48 (28.5%)

Table I. Correlation of cytoplasmic estrogen receptor with clinicopathologic prognostic factors in 168 patients with early endometrial carcinoma

	Estrogen receptor–positive		
	n	%	*p*
Histologic differentiation			
Well differentiated	55	82	<0.01
Moderately differentiated	40	72	
Poorly differentiated	17	46	
Nuclear differentiation grade			
1	38	79	
2	50	79	<0.01
3	26	55	
4	3	33	
Histologic metastases			
No metastases	112	74	
Metastases	6	37	<0.01*
Depth of myometrial invasion			
None	21	68	
Inner third	53	74	0.08
Middle third	24	71	
Outer third	20	65	
Clinical stage			
IA	73	72	
IB	35	71	0.50
II	10	60	
Cervical involvement			
None	100	71	
Mucosa	8	73	0.50
Stroma	10	59	
Peritoneal cytology			
Not performed	6	86	
Negative	82	72	0.30
Positive	30	64	
Histologic category			
Adenocarcinoma	76	75	
Adenoacanthoma	10	71	
Adenosquamous	20	71	0.20
Papillary	9	53	
Clear cell	3	60	

*Fisher's exact two-tail test.

Table II. Correlation of cytoplasmic progesterone receptor with clinicopathologic prognostic factors in 105 patients with early endometrial carcinoma

	Progesterone receptor–positive		
	n	%	*p*
Histologic category			
Adenocarcinoma	47	76	
Adenoacanthoma	6	86	
Adenosquamous	14	78	<0.01
Papillary	3	27	
Clear cell	2	29	
Histologic differentiation			
Well differentiated	33	82	
Moderately differentiated	23	60	<0.05
Poorly differentiated	16	59	
Stage			
IA	42	72	
IB	22	61	0.50
II	8	72	
Nuclear differentiation grade			
1	22	79	
2	27	73	0.30
3	19	78	
4	3	50	
Myometrial invasion			
None	12	75	
Inner third	30	68	0.70
Middle third	16	61	
Outer third	15	75	
Cervical involvement			
None	59	67	
Mucosa	6	75	0.30
Stroma	7	64	
Peritoneal cytology			
Not performed	5	83	
Negative	54	73	0.08
Positive	13	52	
Histologic metastases			
No metastases	64	69	
Metastases	8	67	0.90

grade 3, and nine (5.5%) grade 4 nuclear differentiation. Lesions were subclassified as adenocarcinoma in 101 cases (60.1%), adenoacanthoma in 14 (8.3%), adenosquamous carcinoma in 28 (16.7%), papillary adenocarcinoma in 17 (10.1%), and clear cell carcinoma in eight (4.8%).

The cancer was limited to the endometrium in 31 (18.5%) hysterectomy specimens; 72 (42.9%) had invasion into the inner third, and 65 (38.6%) beyond the inner third of the myometrium. Myometrial invasion extended to within 5 mm of the uterine serosa in 39 (23.2%) of the specimens, while 129 (76.8%) had more than 5 mm of tumor-free myometrium from the serosa. Cervical involvement was not present in 140 (83.3%) hysterectomy specimens, was limited to the endocervical glands in 11 (6.5%), and extended into cervical stroma in 17 (10.1%). Peritoneal washings were analyzed in 161 patients, with 47 (28.6%) having malignant cytology. Pelvic and/or para-aortic lymph nodes were sampled in 130 patients; 14 (10.8%) of these patients had lymph node metastases. Histologic evidence of extrauterine metastases, exclusive of positive peritoneal cytology, was present in 16 (9.5%) patients. All patients were thought to be free of residual disease following primary therapy.

Adjuvant therapy was individualized according to the presence of unfavorable surgical-pathologic features without regard for receptor status. All women in this study had total abdominal hysterectomy with bilateral salpingo-oophorectomy. Pelvic and/or para-aortic selective lymphadenectomies were performed in 130 (77.4%) women. Adjunctive external radiation therapy was given to 40 (23.8%) patients. Postoperative vaginal brachytherapy was used in 18 (10.7%) patients. One patient received preoperative internal and external radiation; steroid receptor analyses were performed before therapy. Intraperitoneal radioactive chromic ^{32}P was utilized in 35 (20.8%) women who had malignant peritoneal cytology. Adjuvant progestin therapy was given to 12 (7.1%) women. Other adjuvant chemotherapy was used in 3 (1.8%) patients.

Associations between clinicopathologic features. As expected, significant relationships were observed between many of the clinicopathologic features previously reported to have prognostic importance for endometrial carcinoma. Histologic differentiation was strongly associated ($p < 0.001$) with nuclear differentiation, extent of myometrial invasion, and histologic documentation of extrauterine metastases. Clinical stage, cervical involvement, and nodal metastases were less strongly associated with histologic differentiation ($p < 0.05$). Of the histologic subtypes of endometrial carcinomas, adenocarcinoma and adenoacanthoma tended to have better differentiated lesions whereas adenosquamous carcinoma, papillary adenocarcinoma, and clear cell carcinoma tended to have moderately or poorly differentiated lesions. Histologic grade was not related to malignant peritoneal cytology or age. Nuclear differentiation was observed to have associations that were similar to those noted for histologic differentiation.

Depth of myometrial invasion was strongly associated ($p < 0.001$) with clinical stage, cervical involvement, nodal metastases, and histologic documentation of extrauterine metastases, in addition to histologic and nuclear differentiation. Depth of invasion was not associated with positive peritoneal cytology ($p = 0.33$). Clinical stage and extent of cervical involvement were both associated with depth of myometrial invasion and histologic documentation of metastases ($p < 0.05$). Age at diagnosis was related ($p < 0.05$) to depth of myometrial invasion but not ($p > 0.05$) with other clinicopathologic features.

The presence of malignant peritoneal cytology was not associated ($p > 0.05$) with histologic grade, nuclear grade, depth of myometrial invasion, clinical stage, or extent of cervical involvement. Seven of 15 (46.7%) of patients with histologic documentation of extrauterine metastases had positive peritoneal cytology compared to 40 of 153 (26.1%) patients with disease histologically confined to the uterus, but this difference was not significant.

Associations of receptor content with clinicopathologic features

Estrogen receptor. Analyses of cytoplasmic estrogen receptors were performed on 168 patients. Binding was determined by sucrose density gradient analysis in 154 cases, while 14 had only dextran-coated charcoal analysis determination of estrogen receptor content. Presence of estrogen receptors was significantly ($p < 0.01$) associated with histologic differentiation, nuclear differentiation, and histologic documentation of extrauterine metastases (Table I). Cytoplasmic estrogen receptor content was not related ($p > 0.10$) to clinical stage, depth of myometrial invasion, cervical involvement, or peritoneal cytology. Fewer estrogen receptor–positive lesions were observed among papillary adenocarcinoma and clear cell carcinoma subtypes, but this trend was not significant.

Table III. Correlation of cytoplasmic estrogen and progesterone receptor status with clinicopathologic prognostic factors in 105 patients with early endometrial carcinoma

	Estrogen receptor and progesterone receptor–positive		
	n	*%*	*p*
Histologic differentiation			
Well differentiated	28	70	
Moderately differentiated	21	55	<0.05
Poorly differentiated	11	41	
Peritoneal cytology			
Not done	4	67	
Negative	47	63	<0.05
Positive	9	36	
Histologic metastases			
No metastases	57	61	
Metastases	3	25	<0.05*
Histologic category			
Adenocarcinoma	38	61	
Adenoacanthoma	6	86	<0.05
Adenosquamous	12	67	
Papillary	3	27	
Clear cell	1	14	
Stage			
IA	36	62	
IB	18	50	0.50
II	6	54	
Nuclear differentiation grade			
1	18	64	
2	24	65	0.10
3	16	48	
4	1	17	
Myometrial invasion			
None	9	56	
Inner third	27	61	0.70
Middle third	12	48	
Outer third	12	60	
Cervical involvement			
None	50	58	
Mucosa	5	62	0.70
Stroma	5	45	

*Fisher's exact two-tail test.

Progesterone receptor. Analyses of cytoplasmic progesterone receptors were performed on 105 of 168 pa-

Table IV. Log-rank analysis of disease-free survival by receptor status and clinicopathologic features

Variable	*Estimated 2 yr survival (%)*	*p*
Estrogen receptor status (n = 168)		
Positive	90	<0.01
Negative	67	
Progesterone receptor status (n = 105)		
Positive	92	<0.001
Negative	56	
Estrogen and progesterone receptor status (n = 105)		
Both positive	94	<0.01
Other	66	
Stage (n = 168)		
IA	92	
IB	88	<0.05
II	64	
Histologic differentiation (n = 168)		
Well differentiated	92	
Moderately differentiated	78	<0.05
Poorly differentiated	72	
Nuclear differentiation grade (n = 168)		
1	88	
2	92	<0.01
3 and 4	68	
Myometrial invasion (n = 168)		
Endometrium only	92	
Inner third	92	<0.001
Middle third	77	
Outer third	54	
Cervical involvement (n = 168)		
None	90	
Mucosa only	100	<0.05
Stroma	28	
Histologic documentation of extrauterine metastasis (n = 168)		
No metastases	88	
Metastases	44	<0.001
Histologic category (n = 168)		
Adenocarcinoma, adenoacanthoma, adenosquamous carcinoma	92	
Papillary adenocarcinoma, clear cell carcinoma	42	<0.01
Peritoneal cytology (n = 161)		
Negative	85	
Positive	69	>0.20

tients; 32 of these determinations were performed by dextran-coated charcoal analysis alone. The remaining 73 specimens were examined by sucrose density gradient analysis. Distribution of progesterone receptors was associated ($p > 0.05$) with histologic subtypes and histologic differentiation (Table II). Clinical stage, nuclear differentiation, depth of myometrial invasion, cervical involvement, peritoneal cytology, and histologic documentation of extrauterine metastases were not associated ($p > 0.10$) with progesterone receptors.

Estrogen and progesterone receptor. One hundred five patients had determination of both estrogen and progesterone receptor status. Cytoplasmic estrogen and progesterone receptor status were significantly ($p < 0.01$) associated: 60 (57.1%) of the lesions were estrogen and progesterone receptor negative, 13 (12.4%) were estrogen receptor positive and progesterone receptor negative, 12 (11.4%) were estrogen receptor negative and progesterone receptor positive, and 20 (19.0%) were estrogen and progesterone receptor negative. The presence of significant estrogen and progesterone receptors was observed to correlate ($p < 0.05$) with histologic differentiation, peritoneal cytology, histologic documentation of extrauterine metastases, and histologic category (Table III). Clinical stage, nuclear differentiation, extent of myometrial invasion, and cervical involvement were not associated ($p > 0.10$) with receptor status. Papillary and clear cell carcinoma subtypes tended to have a much lower proportion of estrogen and progesterone receptor–positive lesions. Only one of 17 (14.4%) and three of 11 (27.3%) of clear cell carcinoma and papillary adenocarcinoma cases respectively had significant estrogen and progesterone receptor binding compared to 56 of 87 (64.4%) of other histologic categories of endometrial carcinoma.

Clinicopathologic features, steroid receptors, and disease-free survival. Since the patient population in this study had early stage endometrial carcinoma and were without evidence of disease following primary therapy, the overall survival was predicted to be good. Therefore, disease-free survival was estimated by means of the Kaplan-Meier life-table and utilized to assess prognosis in these patients. There were 26 recurrences among the 168 patients who had estrogen receptor status determined and 17 recurrences in the subgroup of 105 patients who had both estrogen and progesterone receptor status determined. Overall survival closely paralleled disease-free survival in this study.

When clinicopathologic prognostic variables were analyzed separately, each demonstrated a significant ($p < 0.05$) effect on disease-free survival (Table IV) except for peritoneal cytology. However, the majority of patients with positive cytology received chromic ^{32}P. Cytoplasmic receptor content had a significant effect on disease-free survival: when estrogen receptor status, progesterone receptor status, and combined estrogen receptor/progesterone receptor status were analyzed separately, patients with receptor-positive lesions had

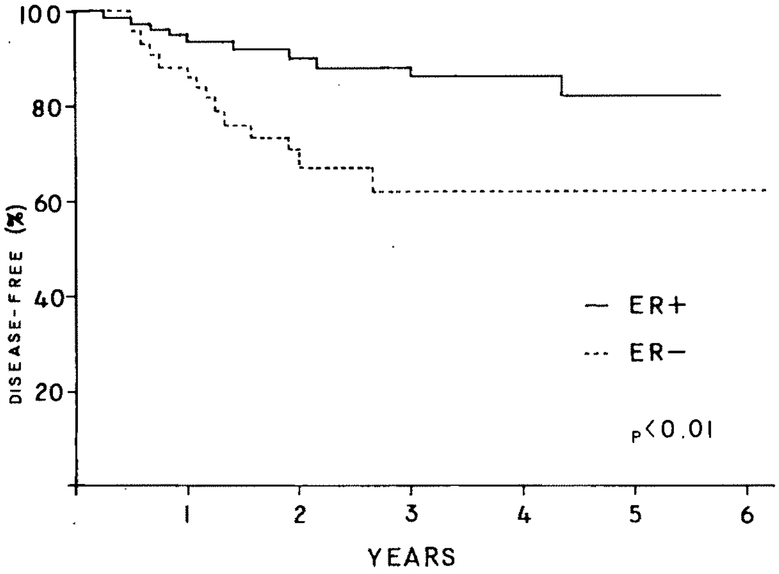

Fig. 1. Disease-free survival in 168 patients with Stages I and II endometrial carcinoma by estrogen receptor *(ER)* status. Patients with estrogen receptor–positive lesions had significantly better prognosis than patients with estrogen receptor–negative lesions ($p < 0.01$).

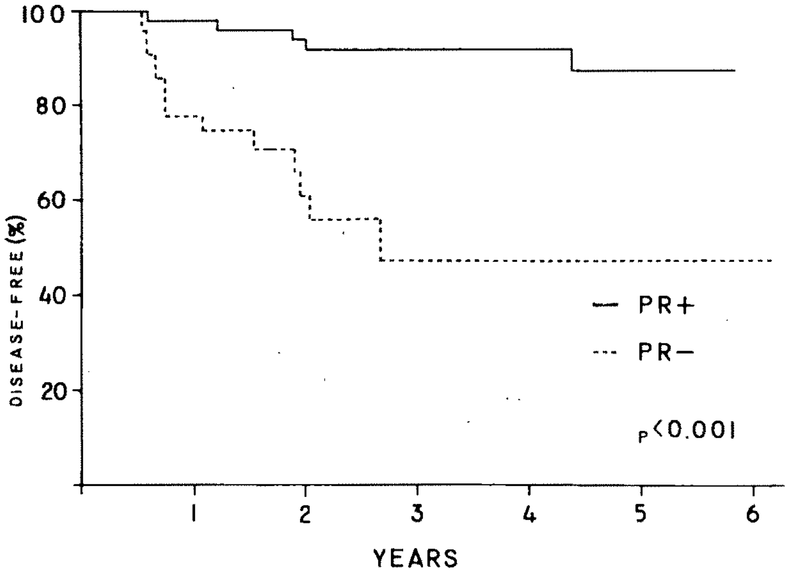

Fig. 2. Disease-free survival in 105 patients with Stages I and II endometrial carcinoma by progesterone receptor *(PR)* status. Patients with progesterone receptor–positive lesions had significantly better prognosis than patients with progesterone receptor–negative lesions ($p < 0.001$).

significantly better disease-free survival than patients with receptor-negative lesions (Figs. 1 to 3). Furthermore, the survival advantage of receptor-positive lesions was retained within every other clinicopathologic category.

Stepwise proportional hazards regression analysis[17] was utilized to assess the relative effects of clinicopathologic and receptor variables on disease-free survival. Receptor status was a significant independent prognostic factor whether defined by estrogen receptor status in the total group of 168 patients and either estrogen receptor/progesterone receptor status or progesterone receptor status alone in the subset of 105 patients with determinations of both receptors. With use of the Cox modeling technique the cervical involvement, age, estrogen receptor status, and extrauterine metastases were the variables selected as describing the likelihood of recurrence among the group of 168 patients with estrogen receptor data (Table V). Age as an univariable factor was not significant in regard to prognosis in either the group evaluated for estrogen receptor or estrogen receptor/progesterone receptor status but did assume importance in the Cox model. Among the 105 patients with both estrogen and progesterone

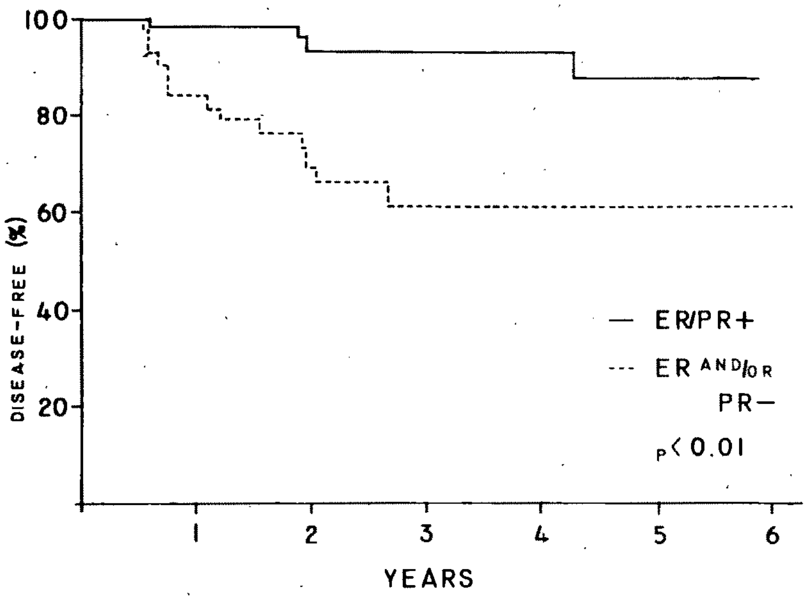

Fig. 3. Disease-free survival in 105 patients with Stages I and II endometrial carcinoma by estrogen receptor and progesterone receptor status. Patients with estrogen receptor/progesterone receptor–positive lesions had significantly better prognosis than patients in whom one or both receptors were negative ($p < 0.01$).

receptor data, both progesterone receptor and estrogen receptor/progesterone receptor status were selected as significant independent prognostic factors (Table VI). When compared to progesterone receptor status, estrogen receptor status alone did not add significant prognostic information to the model in this smaller group of patients. Receptor status remained a significant prognostic factor when the analysis was restricted to patients with disease histologically confined to the uterus. Similar modeling performed without receptor data demonstrated that nuclear differentiation became a significant independent prognostic factor.

Comment

Cytoplasmic steroid receptor content of breast carcinoma is an important independent factor in determining prognosis after primary therapy.[18] Several previous studies have demonstrated relationships between receptor content and histologic differentiation in endometrial carcinomas.[1-3] Although some have suggested an improved prognosis for receptor-positive lesions,[1, 6] no previous study has analyzed estrogen or progesterone receptors as independent prognostic factors when controlling for the effects of conventional histopathologic prognostic factors. In this study, cytoplasmic estrogen and progesterone receptor status of the primary lesions were demonstrated to be significant prognostic factors in Stages I and II endometrial carcinomas.

Steroid receptor status was strongly associated with several known clinicopathologic prognostic factors,[7-10] especially histologic differentiation. With use of receptor status as the sole discriminating factor, the disease-free survival was dependent on receptor status (Figs. 1-3). Disease-free survival was improved for estrogen receptor–positive, progesterone receptor–positive, and estrogen receptor/progesterone receptor–positive lesions when evaluated separately within each clinicopathologic category. When Cox stepwise regression analysis[17] was performed for the entire group of patients, estrogen receptor status was observed to be an important independent variable for predicting survival. Similar modeling for the group of patients who had determinations of both estrogen and progesterone receptors revealed that progesterone receptor and estrogen receptor/progesterone receptor status were essentially equivalent as independent risk factors. When controlled for progesterone receptor status, estrogen receptor status lost prognostic significance in the restricted model. Furthermore, receptor status retained significance as an independent prognostic factor when the model was further restricted to patients with disease limited to the uterus. When receptor status was eliminated from the model, nuclear differentiation became an independent prognostic factor. Therefore, knowledge of estrogen or progesterone receptor status replaced conventional histologic determination of differentiation for predicting time to subsequent recurrence.

As has been previously reported, the estrogen and progesterone receptor content of endometrial carcinomas were related.[1-3] Combined estrogen receptor and progesterone receptor status were strongly related to histologic differentiation: 70% of well-differentiated, 55.3% of moderately differentiated, and 40.8%

Table V. Cox multivariant stepwise proportional hazards model of prognostic factors in 168 patients with cytoplasmic estrogen receptor status

Factor	*Coefficient*	±*SEM*	*p*
Cervical involvement	1.34	0.44	0.0023
Age (linear)	0.05	0.02	0.0092
Extrauterine metastases	0.95	0.48	0.0467
Estrogen receptor–positive status	−1.04	0.43	0.0149

Table VI. Cox multivariant stepwise proportional hazards model of prognostic factors in 105 patients with cytoplasmic estrogen and progesterone receptor status

Factor	*Coefficient*	±*SEM*	*p*
Progesterone receptor–positive status*	−2.43	0.58	<0.0001
Cervical involvement	1.51	0.56	0.0068
Age (linear)	0.07	0.03	0.0130
Extrauterine metastases	1.39	0.59	0.0175

*Estrogen receptor/progesterone receptor status was equivalent to progesterone receptor status in model but lost significance when controlled for progesterone receptor status.

of poorly differentiated lesions were estrogen receptor positive/progesterone receptor positive ($p < 0.05$) whereas 10% of well-differentiated, 18.4% of moderately differentiated, and 33.3% of poorly differentiated lesions were estrogen receptor negative/progesterone receptor negative ($p < 0.05$). Divergence of receptor status (estrogen receptor positive/progesterone receptor negative or estrogen receptor negative/progesterone receptor positive) was less frequently observed and occurred in 20% to 25% of lesions, regardless of histologic differentiation. Significant levels of one receptor with negligible levels of the other may indicate functional defects in normal mechanisms of cellular hormonal regulations.[14] Significant levels of progesterone receptor may be a marker for functional estrogen receptors that are not detected, as has been observed in some breast carcinomas.[18] This may explain the observation that progesterone receptor status or combined estrogen receptor/progesterone receptor status was more important in predicting disease-free survival than estrogen receptor status alone. Unfortunately, all patients in this study did not have determinations of both receptors; therefore this apparent difference may be due to unrecognized bias in the selection of patients who had analysis of both estrogen and progesterone receptors.

Other clinicopathologic features were confirmed to be important prognostic factors for early endometrial carcinoma in this study and were demonstrated to be closely interrelated. The presence of malignant peritoneal cytology was not observed to be a poor prognostic feature in these patients. However, 35 of 47 (74.5%) with positive cytology received intraperitoneal chromic ^{32}P as specific therapy for malignant peritoneal cytology.[9] Additionally, 38 patients with negative peritoneal cytology were excluded from this analysis because of insufficient tumor in the tissue specimen analyzed for receptor status. None of those patients experienced recurrence. Therefore this study does not assess the effect of untreated malignant peritoneal cytology on prognosis in patients with early endometrial carcinoma.

Preliminary data suggest a relationship between cytoplasmic steroid receptor content and response to hormonal therapy of endometrial carcinoma[1, 3, 4] similar to that demonstrated for breast carcinoma. A large proportion of receptor-positive lesions respond to progestin therapy compared to few receptor-negative lesions.[1, 3, 4] It is presently unknown whether the original receptor status remains constant in recurrent and metastatic lesions or whether therapy alters receptor status and function. Knowledge of both estrogen receptor and progesterone receptor status appears to be more discriminant in predicting response to hormonal therapy in breast carcinomas,[18] but sufficient numbers of patients with endometrial carcinomas have not been studied to determine whether knowledge of estrogen receptor and progesterone receptor status is more important than either estrogen receptor or progesterone receptor status alone.

One of the limitations of current steroid receptor technology is the fact that dextran-coated charcoal analysis and sucrose density gradient analysis measure receptor content of whole tissue homogenates. These techniques therefore provide no intrinsic information about the location of estrogen or progesterone receptor in epithelial or stromal components of endometrial carcinomas and contaminating benign tissues. The recent development of a reliable monoclonal antibody specific for human estrogen receptor (H222γSp) and utilizing peroxidase-antiperoxidal staining has allowed quantitative and qualitative identification of estrogen receptor content of various tissue components in endometrial carcinomas. Preliminary evaluation notes an excellent correlation of the monoclonal receptor with sucrose density gradient analysis and dextran-coated charcoal analysis. Future utilization of immunohistologic techniques for determination of estrogen receptor and progesterone receptor status should allow a more precise correlation of receptor content with clinical behavior of endometrial carcinomas.

Based on the results of this study, we recommended that steroid receptor status should be obtained on all primary endometrial carcinomas with the possible exception of grade 1 lesions. Evaluation of receptor content not only allows a better prediction of prognosis than histologic assessment of differentiation, but it also may be helpful in predicting responses to therapy at the time of a future recurrence. Further studies evaluating the effect of adjuvant therapy in primary treatment of high-risk patients with endometrial carcinomas should take into account receptor status as an independent prognostic factor. Finally, study of cytoplasmic steroid receptor content allows insight into the cellular regulation of malignancies and may help to explain why individual lesions do not always behave in a manner predicted by conventional histopathologic techniques.

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Discussion

DR. SAUL B. GUSBERG, New York, New York. I am pleased with the opportunity to discuss this excellent paper by Dr. Creasman, the Drs. McCarty, and their colleagues that presents the steroid receptor status of endometrial carcinoma as an independent variable with important prognostic value. The steroid biochemistry is carefully done, as one expects from these laboratories at Duke University, and the statistical treatment of results is sophisticated, enabling the assayer to bring relatively small numbers to significance.

My pleasure with this study does not mean that I have failed to note some interesting and unusual aspects that seem to require clarification: (1) The use of the term "early clinical stage" refers, I presume, only to the International Federation of Gynaecology and Obstetrics stage, for biologically these tumors may be advanced while remaining clinically local. (2) The term "no residual disease," given 10.8% positive nodes and 28.6% with malignant cells in peritoneal washing, clearly must refer to gross macroscopic disease. (3) "Cytoplasmic receptor" must mean cytosol receptor, the soluble fraction of the cytoplasm after the nucleus, membranes, Golgi, etc., are spun down. (4) Selective lymphadenectomies were performed in 130 of these 168 patients, suggesting that some were performed as a routine matter rather than for specific cause.

While it is clear that some subgroup numbers are too small to express as percentages, it is notable in this series that only 40% of the whole group are described as well differentiated, which seems low for this disease, while the so-called virulent group, including adenosquamous, papillary, and clear cell tumors, constitute 31.6%, which seems high. I am among those who have problems distinguishing some of these subgroups histologically in my own material.

One cannot quarrel with the use of 10 to 15 fmol of steroid receptor per milligram of protein as the cut-off point between negative and positive, but one should recognize this as an arbitrary standard, for some studies use lower points of distinction and others use 50 fmol as possibly in greater conformity with the necessities of normal cycling tissue change. Indeed, the last word about steroid metabolism and receptor technology has not been spoken, for considerations of nuclear binding, enhancement of binding by cyclic nucleotides, norepinephrine, and melatonin, and decline with insulin have been reported in current studies.[1] State-of-the-art technology must be used, of course, for clinical investigation, and that has been done well here.

I regard this study as a significant contribution, not so much for the finding that receptor status (particularly progesterone receptor positive and estrogen receptor and progesterone receptor positive) is an independent variable for prognosis; the power of histologic differentiation, in this respect, is about as great, easier to assay, and less expensive technically, and even obesity may be a respectable prognosticator. However, I regard the finding that 40.8% of the poorly differentiated tumors were estrogen receptor positive/progesterone receptor positive and that some with deep myometrial invasion and some papillary and clear cell tumors were receptor positive as very important for treatment. Progesterone receptor studies to date have frustrated some of us eager for a new approach to the more malignant carcinomas of the endometrium, for in general they have shown the presence of receptor in the more easily-cured, well-differentiated tumors and the absence of receptor in the undifferentiated, with clear correlation of the benefit of hormonal treatment with receptor presence. Dr. Creasman's study, on the other hand, offers hope for improved treatment of these more difficult problem patients, and the new-found ability to manipulate progesterone receptor supplements this hope. In this carefully studied cohort of patients it is a most significant finding.

Finally, I have been told that total happiness requires perfect health and no memory. Unfortunately, I do not qualify. I was not surprised by the result reported in this fine study, for one must expect that tumor tissues that share the biochemical properties of the host tissue will be less virulent. In simpler times by simpler cytochemical methods we demonstrated this phenomenon some years ago, and we referred to the difference between dependent tumors and autonomous tumors.[2] I mention this instance of déjà vu only to congratulate Dr. Creasman and to offer him another reference for his bibliography.

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Dr. Robert B. Jaffe, San Francisco, California. I have two endocrinology questions that this paper raised.

I would like to remind the audience of the work of Gerald Cunha, who has demonstrated that while the end result of steroid action may be on the epithelium, that action may be exerted via the mesoderm. Therefore, we should not look only at the epithelium for the mechanism of action of these steroids.

In regard to cytoplasmic receptors, Dr. Siiteri in the 1970s demonstrated, and it has been recently confirmed by two other outstanding laboratories, that there may be very little physiologic significance of receptors in the cytoplasm, and that it is the nuclear receptors for steroids which really are the major receptors that need to be borne in mind.

Dr. Seymour Romney, New York, New York. Are there any data in your study which would allow us to give all patients who have endometrial carcinoma, possibly with the exception of Stage I, grade 1 lesions, some progesterone either before or after instituting primary therapy, bearing in mind that progesterone may be helpful in inducing estrogen receptors?

Dr. Arthur L. Herbst, Chicago, Illinois. Based on studies at the Ben May laboratory in our institution and also on some work in our department looking at monoclonal antibodies by means of both light microscopy and ultrasound techniques, these antibodies, as Dr. Creasman pointed out, will react against the steroid receptors in endometrial tissue and also against some of the receptors that are in some of the ovarian epithelial tumors.

What this seems to show is not only heterogeneity of the tumor tissue but also that the localization of the staining is almost completely within the nucleus, so that much of what we have been calling cytoplasmic receptor for years may, in fact, not be cytoplasmic but just nuclear receptor that has shaken loose during the homogenization process.

Dr. Carmel J. Cohen, New York, New York. The 40% receptor-positive distribution in the grade 3 lesions is a finding that I found surprising. Was the survival rate in the grade 3 patients a function of receptor positivity? The second question is: Do you have any data on receptor changes in any of the recurrences?

Dr. J. Donald Woodruff, Baltimore, Maryland. I noted that there was a relationship between age and survival in this study. I wonder if there is any difference in the histology of these tumors because there is more than one type of endometrial cancer. There is one type that occurs in women in the menopausal and perimenopausal years; this type is estrogen dependent. The one that occurs in women in the seventh and eighth decade is usually poorly differentiated and probably has a different basis for its genesis.

Was there any difference in the tumor type as related to the age of the patient?

Dr. Samuel Solomon, Montreal, Quebec, Canada. The basis for doing progesterone and estrogen receptor studies is to try to do the same thing for the endometrium that has been done for the breast. If you look at the history of breast tissue receptor studies, the difficulties seen in this study were seen earlier in the breast studies.

Although the pharmacologists disagree, I have always viewed receptors as operational terms. No one has ever isolated a receptor. You make receptors by spinning tissues as Dr. Jaffe said, and by doing so, you may dislodge them or you may make a fraction that is present in both the cytoplasmic soluble and in the nucleus. But for the sake of these studies, it makes no difference whether it is biologically significant or not, because what you are trying to do is to get a number that you can relate to prognosis. In the case of the breast studies, that has worked out very well.

The cutoff is not important either, because in the breast studies we used to argue about 5 fmol per milligram of protein versus 20 fmol as a cutoff. Some investigators used 6 fmol, but in the end it made no difference because we realized later that if you had a value below 30 fmol, you were in trouble with a primary breast cancer, and that was the important finding.

I urge you to press on and, if you are going to use monoclonal antibodies, see whether you can raise your own antibodies against endometrial receptors. Do not use breast tissue, because that may not be the same.

Dr. Creasman (Closing). Several of the questions raised were related to methodology and terminology. The International Federation of Gynaecology and Obstetrics staging was used. We took some liberty with some definitions, although still strictly following the clinical stage (FIGO) but possibly not surgical staging. We stated that there was no disease remaining after surgery if gross disease was not present even if there were patients with malignant cells in the peritoneal cytology or metastasis to the lymph nodes. I think four or five patients with positive lymph nodes but also with residual gross disease were excluded from this study.

A comment was made concerning the fact that only three-fourths of our patients had lymphadenectomies. From previous studies, we noted that only 1% to 2% of grade 1 lesions had lymph node metastasis; although at one time we did selective lymphadenectomy on these patients, we now limit this to those with grade 2 and 3 lesions only.

Forty percent of our patients did have grade 1 lesions; this is about what we have seen in previous data from our institution. Since our practice is a referral one, this probably accounts for a lower figure than reported by other authors.

The problem with subtypes is certainly very evident. We asked two independent pathologists to review all of the pathologic material. For papillary and clear cell tumors at least 50% of the tumor was required to have that particular cell component in order to be classified in those categories.

Your remark about our level of femtomoles required to qualify as "positive" is certainly pertinent. Our steroid receptor laboratory, run by the Drs. McCarty, has had a long experience with breast as well as gynecologic malignancies and the femtomole levels chosen for positive and negative level are based on this extensive experience.

An appropriate question was raised as to whether or not receptor status was really more useful than differentiation with regard to prognosis or was just another additional bit of information. In this study, nuclear grade was much more important than FIGO grade. In many pathology laboratories even FIGO grade is not routinely given on the pathology report. I think it would be somewhat naive to believe that we will get a nuclear grade; therefore receptor analysis may be the answer. The cost of receptor analysis is actually less than what is charged for the pathology analysis, which is something that should be considered. Receptors are a better discriminator than is grade.

Several comments were made concerning the receptor technique itself. As noted in the manuscript, cytosol cytoplasmic receptors were evaluated in the study. It is true the effects of receptors are probably related to their nuclear activity. Certainly the staining we are seeing with the monoclonal technique is nuclear and not cytoplasmic. These newer techniques are going to change, we think, our whole concept of receptor technology. We will be able to evaluate the heterogeneity of tumors among other things.

There was a remark, at least intimated, about manipulating hormone receptors. We have been evaluating this possibility in the nude mouse model. Estrogen receptor–negative tumors have been made estrogen receptor–positive with exogenous hormones. We feel this may have tremendous implications, not only in endometrial cancer, but possibly in ovarian cancer which also has receptors. In this latter malignancy we need all the help we can get. If we can manipulate receptors and take this capability from the laboratory to the patient, then we may be able to make a tremendous stride forward.

Dr. Cohen asked about the 40% positive receptors in grade 3 lesions and whether this related to survival. The current data are still being analyzed, but in previous studies as well as preliminary evaluation of this material, receptors did predict survival even within the poorly differentiated lesions.

We have some information concerning receptor data and recurrent lesions. Although the numbers are small, we have a >90% positive relationship with regard to hormone (progestin) therapy response and receptor status.

Dr. Woodruff asked about age as a prognostic factor. As a univariable factor it did not appear to be important but became significant when applied to the Cox (multivariant) analysis. The relationship of histologic subtype and prognosis appear to be more discriminated when receptors are also considered.

Because her estrogen therapy shouldn't affect her blood pressure

In a three-month study,[1] none of the 184 patients receiving OGEN 1.25 experienced a significant rise in BP. About 15% of the 144 patients receiving conjugated estrogens (0.625 mg) experienced a significant rise of at least 15 mm Hg. That's a difference that could make a real difference for your patients who may be hypertensive or at risk of developing hypertension.

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e adjacent page for brief summary.

ference:
Wren BG, Routledge AD: The effect of type and dose of oestrogen on the blood pressure of post-menopausal women. *Maturitas* 5:135-142, 1983.

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Only estrone. Identical to the body's own.

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ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods.[1-3] This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.[4]

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment[1] and on estrogen dose.[3] In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration;[3] it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.[5,6] In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures.[7] Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis,[8-12] epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects.[13-16] One case control study[16] estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipete tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS

OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. Known or suspected pregnancy (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,[17] although a recent long-term followup of a single physician's practice has raised this possibility.[18] Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens,[17] similar to the 2-fold increase previously noted in users of oral contraceptives.[19,22] In the case of oral contraceptives, the increased risk appeared after two years of use.[22]

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.[20,21]

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.[22-29] Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug.[30,31] An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives.[32,33] If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found[17,34] this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men[35] to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives.[36-38] Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives.[37] The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause[39] and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression.[22] Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

10. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

11. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. OGEN (estropipate tablets) cannott be recommended for use during pregnancy. See Contraindications and Boxed Warning.

D. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Breakthrough bleeding, spotting, change in menstrual flow.
Dysmenorrhea.
Premenstrual-like syndrome.
Amenorrhea during and after treatment.
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Change in cervical eversion and in degree of cervical secretion.
Cystitis-like syndrome.

2. *Breast.*
Tenderness, enlargement, secretion.

3. *Gastrointestinal.*
Nausea, vomiting.
Abdominal cramps, bloating.
Cholestatic jaundice.

4. *Skin.*
Chloasma or melasma which may persist when drug is discontinued.
Erythema multiforme.
Erythema nodosum.
Hemorrhagic eruption.
Loss of scalp hair.
Hirsutism.

5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.

6. *CNS.*
Headache, migraine, dizziness.
Mental depression.
Chorea.

7. *Miscellaneous.*
Increase or decrease in weight.
Reduced carbohydrate tolerance.
Aggravation of porphyria.
Edema.
Changes in libido.

ACUTE OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

The usual dosage range is one OGEN .625 (estropipate) Tablet to one OGEN 5 Tablet per day.

2. *Given cyclically:*

Female hypogonadism. Female castration. Primary ovarian failure. The lowest dose that will control symptoms should be chosen. A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The same schedule is repeated if bleeding does not occur by the end of the rest period. The duration of cyclic estrogen therapy necessary to produce withdrawal bleeding will vary according to the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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	SPOTTING	BREAKTHROUGH BLEEDING
Cycle 1	10.6%	8.8%
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Lo/Ovral® offers less breakthrough bleeding and spotting, along with high contraceptive efficacy, at a low dose—just 30 mcg ethinyl estradiol and 0.3 mg norgestrel.

LOW DOSE

LO/OVRAL® 30 mcg

each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol, Wyeth.

1. Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.

*Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.

†Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

‡Data on file for 22,489 total cycles, Wyeth Laboratories. **See important information on following page.**

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5- or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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The management of fetal hydrocephalus

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The management of 53 consecutive documented cases of fetal hydrocephalus is presented. Hydrocephalus was isolated in nine cases (17%) and associated with other abnormalities in 44 (83%). Our success in diagnosing abnormalities associated with hydrocephalus improved from the time period 1977 through 1981 to that of 1982 through 1983. In this series, management consisted of termination of pregnancy, 14 (26%); ventriculoamniotic shunt placement and cesarean section, two (4%); cephalocentesis and cesarean section, two (4%); cesarean section without cephalocentesis or ventriculoamniotic shunt placement, 18 (34%); cephalocentesis and vaginal delivery, nine (17%); and vaginal delivery without cephalocentesis, eight (15%). The outcomes were spontaneous abortion, 14 (26%); intrapartum death, eight (15%); postnatal death, 16 (30%); currently alive, 15 (28%). Current approaches to the management of fetal hydrocephalus are discussed, and areas of therapeutic uncertainty are delineated. (AM J OBSTET GYNECOL 1985;151:933-42.)

Key words: Hydrocephalus, antenatal diagnosis, ultrasound

The diagnosis and management of fetal hydrocephalus has long challenged the physician. Although both Hippocrates and Galen described cases of hydrocephalus, Vesalius, in 1550 AD, was the first to recognize that large amounts of fluid could accumulate inside the brain.[1] In the nineteenth century, hydrocephalus was recognized as a rare, but potentially catastrophic, complication of pregnancy. In 1887, Charpentier[2] described that among 200 cases of congenital hydrocephalus, 48 maternal deaths resulted. Decompression of the enlarged fetal head was necessary to save the mother's life, by averting the complications of obstructed labor or cesarean section. Although the maternal morbidity and mortality of cesarean section have been greatly reduced, intrapartum cephalocentesis is still used for selected cases of fetal hydrocephalus.

Recently, another option has emerged in the management of fetal hydrocephalus. Advances in diagnostic imaging have enabled detection of fetal hydrocephalus prior to fetal viability, thereby permitting the option of termination of pregnancy.[3]

Improvements in the outlook for congenital hydrocephalus have also influenced obstetric management. Normal, and even superior, intelligence has been reported for some hydrocephalic infants, especially those without associated abnormalities, who have been given aggressive neurosurgical care.[4, 5] Consequently, atraumatic delivery by cesarean section has been recommended for some cases of hydrocephalus.[6] In addition, intrauterine decompression of dilated cerebral ventricles has been performed in an attempt to reduce the ill effects of sustained cerebral compression from unrelieved hydrocephalus. Repeated needle aspiration[7] and ventriculoamniotic shunts[8] have been used for this purpose.

Our group has recently reported our experience with the diagnosis of fetal hydrocephalus.[9] While the diagnostic accuracy of antenatal ultrasound in the detection of fetal hydrocephalus was established, the consistent identification of abnormalities associated with this condition was not as successful. The purpose of this communication is to report our experience with the management of fetal hydrocephalus and to update our accuracy with the detection of associated abnormalities. Our aim is to discuss current approaches to the management of fetal hydrocephalus and to delineate areas of therapeutic uncertainty, based on our experience with 53 consecutive documented cases.

Material and methods

Sixty-two fetuses were diagnosed at the Perinatal Ultrasound Unit of Yale–New Haven Medical Center as having hydrocephalus during the 6-year period from November, 1977, to November, 1983. This represents our complete experience (as no cases were omitted). Hydrocephalus was determined to be present if the lateral ventricle–to–hemispheric width ratio was abnormal as defined by the nomogram of Jeanty et al.[10]

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Table I. Antenatal detection of abnormalities in association with hydrocephalus

	Antenatal diagnosis, isolated hydrocephalus	*Antenatal diagnosis, hydrocephalus with abnormalities*
Postnatal diagnosis, isolated hydrocephalus	6,* 3†	0,* 0†
Postnatal diagnosis, hydrocephalus with abnormalities	10,*‡ 4†§	10,* 20†

*Cases of fetal hydrocephalus diagnosed during 1977 through 1981.

†Cases of fetal hydrocephalus diagnosed during 1982 and 1983.

‡Anomalies not diagnosed antenatally: lumbosacral spina bifida (four), tetralogy of Fallot (one), ring 18 chromosome (one), sirenomelus (one), clubfoot (one), tracheoesophageal fistula (one), hypoplastic digits (one).

§Anomalies not diagnosed antenatally: sacral spina bifida (one), agenesis of corpus callosum (one), small ventricular septal defect (one), congenital toxoplasmosis infection (one).

Table II. Management of 53 cases of documented fetal hydrocephalus

	Abortion	*Intrapartum death*	*Postnatal death*	*Alive*	*Total*
Termination of pregnancy	14	–	–	–	14 (26%)
Cesarean section					
Ventriculoamniotic shunt and cesarean section	–	–	–	2	2 (4%)
Cephalocentesis and cesarean section	–	–	2	–	2 (4%)
Cesarean section (other)	–	–	7	11	18 (34%)
Vaginal delivery					
Cephalocentesis and vaginal delivery	–	6	3	–	9 (17%)
Vaginal delivery (other)	–	2	4	2	8 (15%)
Total	14 (26%)	8 (15%)	16 (30%)	15 (28%)	53 (100%)

If the lateral ventricle–to–hemispheric width ratio could not be determined, then the diagnosis was made by the presence of marked and obvious dilatation of the ventricles. In those cases with adequate follow-up, there were no false positive diagnoses of hydrocephalus in this series.

In nine cases, delivery occurred at outlying hospitals, and hydrocephalus was not documented because of failure to perform a careful postmortem examination of the neonatal brain. There were no survivors in this group. The 53 cases in which an autopsy or neonatal diagnostic imaging examination was performed and hydrocephalus was documented are the subject of this report.

The 26 cases which were diagnosed between 1977 and 1981 were retrospectively reviewed for diagnosis, management, and outcome. The 27 cases detected during 1982 and 1983 were prospectively collected.

Abnormalities associated with hydrocephalus were reported to be present if a structural defect or chromosomal aberration was identified. However, structural defects directly attributable to the effects of hydrocephalus (for instance, thinning of cerebral cortex) were not included as associated abnormalities. In addition, toxoplasmosis infection with multiple organ involvement, which was identified during the postmortem examination in one case, is included as an associated abnormality.

Results

The 26 cases of fetal hydrocephalus, detected during 1977 through 1981, had a mean gestational age of 31.5 weeks at the time of diagnosis. Twenty-seven cases, detected during 1982 and 1983, had a mean gestational age of 28 weeks at the time of diagnosis. Of the 53 cases, hydrocephalus was an isolated defect in nine (17%) and associated with other abnormalities in 44 (83%). Spina bifida, the most common associated abnormality, occurred in 15 cases. Associated abnormalities were also present in the neurological, cardiovascular, renal, gastrointestinal, skeletal, respiratory, and reproductive systems. There were five chromosomal aberrations. These were one case each of trisomy 18, trisomy 21, ring chromosome 18, mosaic (46,XY/48,XY, +7, +8), and balanced translocation (long arm chromosome 12 and short arm 5).

Our experience with the antenatal detection of abnormalities in association with hydrocephalus is shown in Table I. In the entire group, there were 14 cases where the antenatal diagnosis of isolated hydrocephalus was incorrect as other abnormalities were identified in the postnatal period. Of these, 10 cases were not correctly identified during 1977 through 1981: lumbosacral spina bifida (four), tetralogy of Fallot (one), ring 18 chromosome (one), sirenomelus (one), clubfoot (one), tracheoesophageal fistula (one), and hypoplastic digits (one). Four cases were not correctly identified

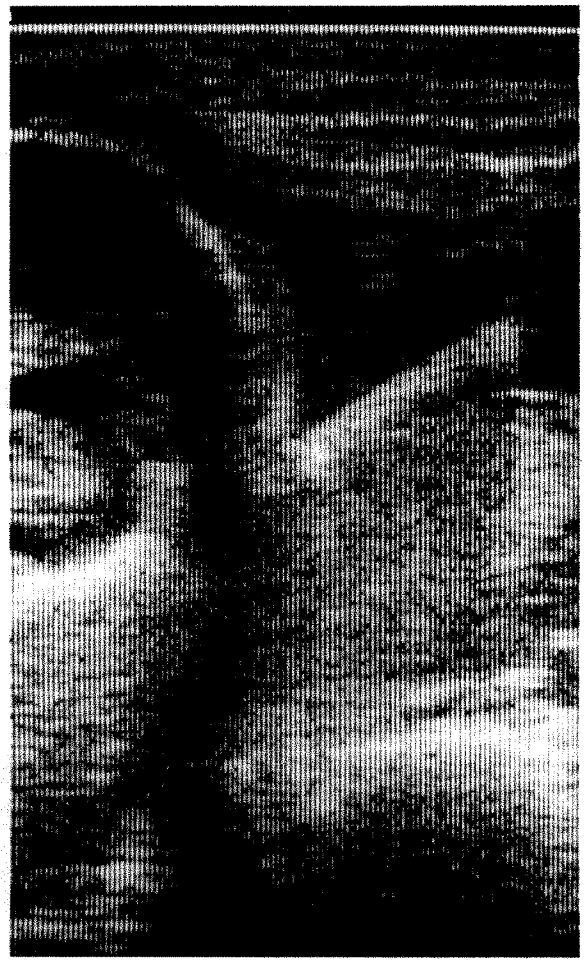

Fig. 1A. Cranial sonogram demonstrating ventriculoamniotic shunt protruding from fetal skull.

during 1982 and 1983: sacral spina bifida (one), agenesis of corpus callosum (one), small ventricular septal defect (one), and congenital toxoplasmosis infection (one).

The obstetric management of the 53 cases is summarized in Table II. In 14 cases (four in 1977 through 1981; 10 in 1982 and 1983), the pregnancies were terminated when the hydrocephalus was diagnosed prior to fetal viability. Of the remaining 39 cases, cesarean sections were performed in 22 (56.4%) and vaginal delivery occurred in 17 (43.6%).

Ventriculoamniotic shunts were placed in two fetuses. The shunt system was valveless, constructed of sterile silicone rubber tubing with a maximum outer diameter of 2.2 mm, and was inserted through a 12-gauge needle during local anesthesia.

In the first case, fetal hydrocephalus was diagnosed at 26 weeks of gestation during an ultrasound examination for gestational age assessment. No other abnormalities were identified and the fetal karyotype was 46,XY. A ventriculoamniotic shunt was placed through the lateral aspect of the fetal occiput at 27½ weeks of gestation after progressive hydrocephalus was demonstrated. The shunt was observed by both radiograph and sonogram to be properly placed (Fig. 1A). There was sonographic evidence of decompression of the hydrocephalus from the day after shunt insertion to 4½ weeks after insertion. At 6½ weeks, however, ventriculomegaly worsened, indicating shunt failure during this 2-week period. Because of the worsening hydrocephalus, an amniocentesis was performed at 35 weeks

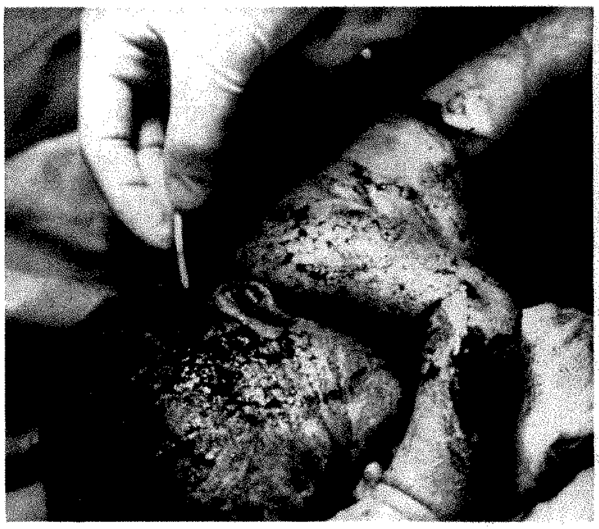

Fig. 1B. Ventriculoamniotic shunt protruding from skull of newborn infant.

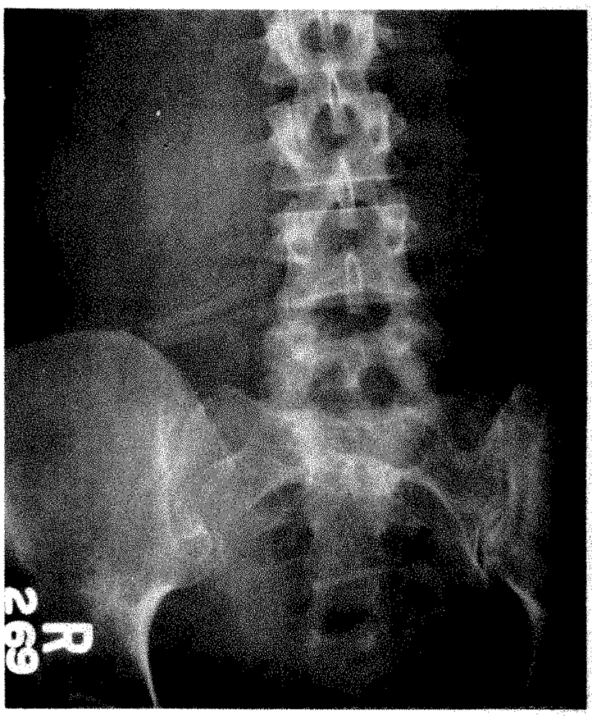

Fig. 1C. Radiograph demonstrating ventriculoamniotic shunt entering fetal skull *(arrow)*.

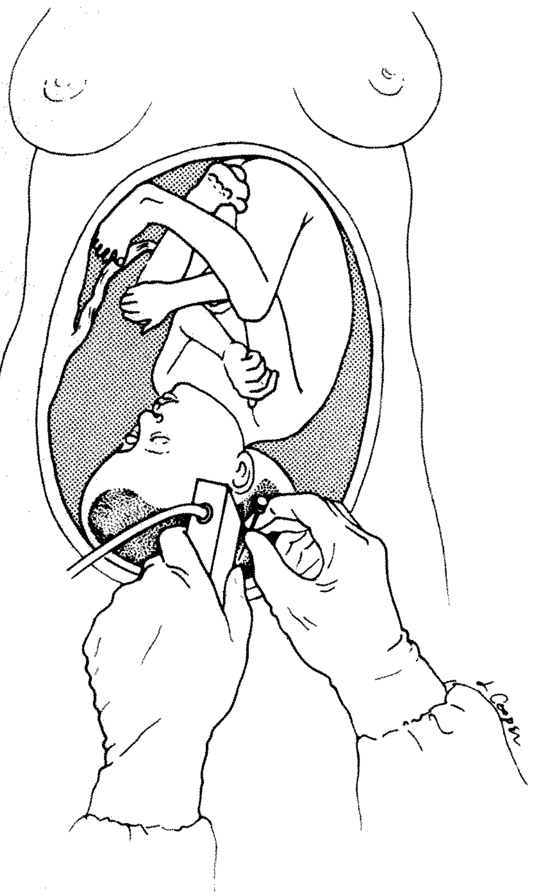

Fig. 2. Method of cephalocentesis. An 18-gauge needle is placed into fetal skull through the maternal abdomen with real-time sonographic guidance.

of gestation and the lecithin/sphingomyelin ratio was 1.2. Betamethasone was given to the mother prior to an elective low vertical cesarean section. A 2340 gm male infant with Apgar scores of 9 at 1 minute and 9 at 5 minutes was delivered, and the shunt was removed from the fetal head shortly after birth (Fig. 1B). The postnatal course was complicated by mild respiratory distress syndrome. Physical examination revealed hypoplastic digits of both the upper and the lower extremities. A ventriculoperitoneal shunt was placed on the third day of neonatal life. Developmental status at 10 months of age revealed moderate mental and motor developmental delays (Bayley mental score, 69; Bayley motor score, 73).

In the second case, fetal hydrocephalus was diagnosed at 28 weeks of gestation during an ultrasound examination for gestational age assessment. A large midline arachnoid cyst was present on sonography and the karyotype of amniocytes was 46,XX. A ventriculoamniotic shunt was placed through the lateral aspect of the fetal occiput at 29½ weeks of gestation after progressive hydrocephalus was demonstrated. Both radiograph (Fig. 1C) and sonogram demonstrated the shunt to be properly placed. Shunt function was uncertain as head size and ventriculomegaly slowly increased after placement. A low vertical cesarean section was performed at 37 weeks of gestation after pulmonary maturity was demonstrated, with delivery of a 2730 gm female infant with Apgar scores of 9 at 1 minute and 9 at 5 minutes. The ventriculoamniotic shunt was not evident at birth, and a neonatal skull film showed it to be entirely intracranial. Neonatal axial computed tomography revealed hydrocephalus in association with a large midline cyst and dysgenesis of the corpus callosum. Three days after delivery, a ventriculoperitoneal shunt was placed, and the retained ventriculoamniotic shunt was removed uneventfully. Developmental status at 9 months of age revealed severe mental and motor developmental delays (Bayley mental score, <50; Bayley motor score, <50).

Cephalocentesis was performed in 11 cases. Nine of these were done in order to permit vaginal delivery of an enlarged head in nine pregnancies. Two were performed to permit delivery through a low transverse uterine incision in women with a history of previous cesarean section. The amount of cerebrospinal fluid removed varied from 150 to 1700 ml. As these procedures were performed by different operators at several institutions, there was no uniformity of technique.

In 10 cases of cephalocentesis, perinatal death was associated with the procedure; six were intrapartum deaths and four were neonatal deaths within 3 hours of delivery. In one instance where 150 ml of fluid was removed, perinatal death was not associated with cephalocentesis. This infant, however, died at 2 months of age of respiratory laryngitis. Of the 11 cases of cephalocentesis, autopsy evidence of intracranial bleeding was present in seven and sonographic visualization of intracranial bleeding was observed in three.

In 18 additional cases (excluding the two ventriculoamniotic shunts and two cases in which cephalocentesis was performed), delivery of the hydrocephalic infant was accomplished by cesarean section. Cesarean section was elective in 12. For these 12, indications were hydrocephalus with macrocephaly (five cases), hydrocephalus with normal-sized head associated with spina bifida (four cases), worsening hydrocephalus with normal-sized head and an unfavorable cervix (one case), hydrocephalus with a large intracranial teratoma and macrocephaly (one case), and hydrocephalus with macrocephaly associated with an encephalocele in a patient with a previous low vertical cesarean section (one case). In nine of these 12 elective cesarean sections, fetal lung maturity was documented with a lecithin/sphingomyelin ratio >2.0. In two cases, gestational age based on menstrual history and early sonographic examination was >38 weeks in nondiabetic patients. In the twelfth patient, elective cesarean section was performed

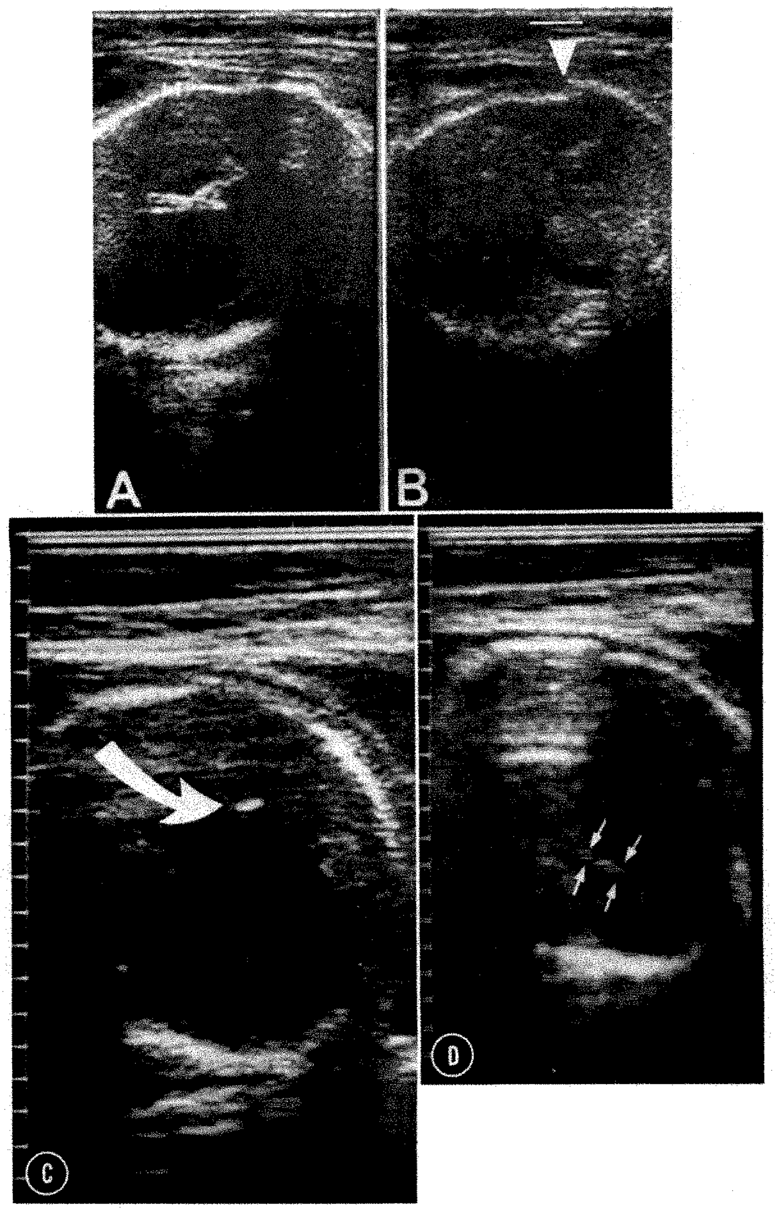

Fig. 3. *A*, Sonogram of fetal head prior to cephalocentesis. *B*, Sonogram of fetal head after cephalocentesis with arrow pointing to overlapping cranial sutures. *C*, Sonogram of fetal head with arrow pointing to tip of 18-gauge needle in dilated ventricle. *D*, Sonogram of fetal head after cephalocentesis, with arrows outlining stream of blood.

at 34 weeks of gestation because of increasing ventriculomegaly and head size. In this case, the lecithin/sphingomyelin ratio was 1.7, betamethasone was given to the mother prior to delivery, and there was no respiratory distress after birth.

In the six remaining cases, cesarean section was performed following the onset of spontaneous labor. Indications for cesarean section were hydrocephalus with macrocephaly (three cases), hydrocephalus with normal-sized head associated with spina bifida (two cases), and prematurity and breech presentation in a fetus with hydrocephalus and normal-sized head (one case).

Three of the 18 infants delivered by cesarean section died shortly after birth as a result of severe associated anomalies. Four neonatal deaths were due to respiratory insufficiency or symptomatic Arnold-Chiari malformation, and one of these was a postoperative death after repair of an intracranial arteriovenous malformation. In one case, delivery was at a referring hospital where severe isolated hydrocephalus was managed with no neurosurgical intervention, and death occurred 10 days after delivery. Eleven infants in this group are currently alive.

Vaginal delivery occurred in eight cases in which

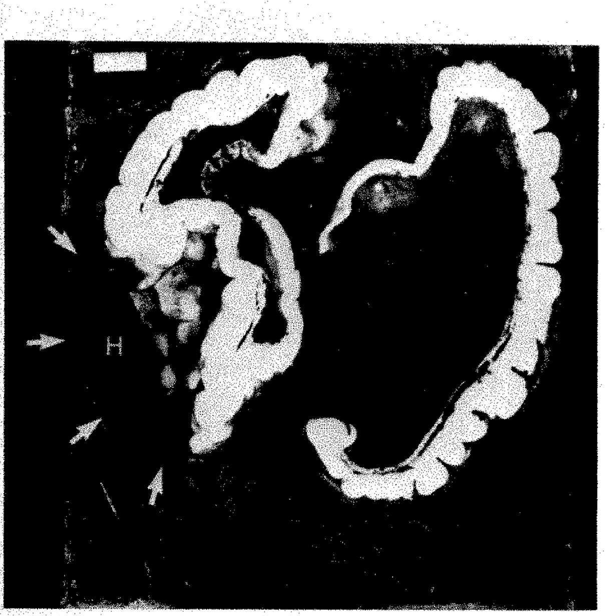

Fig. 4A. Coronal section of brain demonstrating a large subarachnoid hemorrhage *(H)* resulting from cephalocentesis. (Modified from: Isaacson G. Postmortem examination of infant brains. Arch Pathol Lab Med 1984;108:80.)

cephalocentesis was not performed. Labor was spontaneous in seven of these cases and induced in one case of hydrocephalus not associated with macrocephaly. Intrapartum death occurred in two fetuses when fetal heart rate monitoring was not used. Neonatal death occurred less than 2 hours after birth in four infants with severe anomalies associated with hydrocephalus. The remaining two infants delivered vaginally are currently alive.

Comment

This series confirms our previous finding[9] that fetal hydrocephalus usually occurs in association with other abnormalities. Our success in identification of them in association with hydrocephalus improved from the time period 1977 through 1981 to 1982 through 1983. In the former period, 10 of the 26 cases (38%) of hydrocephalus with other abnormalities were not correctly identified, while in the latter 2 years, this was true for four of the 27 cases (15%). The realization that associated abnormalities could affect any body organ system was most likely responsible for this improvement. In addition, more intensive, earlier efforts at antenatal diagnosis and improved diagnostic imaging also contributed to this finding. It is clear that a meticulous sonographic examination should always be performed and karyotypic evaluation of fetal amniocytes should also be considered when fetal hydrocephalus is detected antenatally. In addition, amniotic fluid α-fetoprotein determination and viral cultures may be useful.

Even with these efforts, however, every associated abnormality cannot be reliably detected at present. During the years 1982 and 1983, abnormalities with hydrocephalus were not detected antenatally in four cases. In one, a sacral spina bifida in a term gestation was not identified. Fetal position and decreased amniotic fluid probably contributed to this diagnostic error. For the other three abnormalities (agenesis of the corpus callosum, small ventricular septal defect, and congenital toxoplasmosis infection), failure of detection was not due to technical error. In these latter cases, our inability to appreciate the abnormalities was due to current inadequacies in the art of antenatal diagnosis. It is anticipated that with further developments, our ability to discriminate isolated fetal hydrocephalus from hydrocephalus in association with other abnormalities will improve. Further efforts at antenatal diagnosis should endeavor to identify all abnormalities in association with fetal hydrocephalus as even minor structural abnormalities may be markers of a brain dysgenesis more severe than isolated ventriculomegaly.[5]

The obstetric management of fetal hydrocephalus is dependent upon gestational age at the time of diagnosis and the association of other anomalies. Therapeutic options include termination of pregnancy prior to fetal viability, ventriculoamniotic shunt placement, cephalocentesis prior to delivery, and/or cesarean section.

The therapeutic value of ventriculoamniotic shunt placement is uncertain at the present time. Although experimental placement of these shunts in fetal rhesus monkeys yielded encouraging results,[11] clinical experience in human pregnancies is limited,[8, 12] and long-term follow-up of postnatal neurological outcome is lacking. Two fetuses in this series were treated with ventriculoamniotic shunts. In one, the device appeared to be functional for at least 4½ weeks, while, in the other, the timing of intracranial shunt migration was unclear. Neither neonate is developing normally, but it is uncertain how they would have fared if in utero shunting had not been performed.

Further clinical investigation is obviously necessary before the potential benefits and the potential harms of ventriculoamniotic shunt placement can be weighed, and the value of this procedure in the management of fetal hydrocephalus definitely determined. At the present time, if ventriculoamniotic shunt placement is ever considered, it should be limited to those cases of isolated hydrocephalus where progressive ventriculomegaly has been documented and the fetus is too immature to be delivered for postnatal shunting. A multidisciplinary team comprising a perinatologist, ultrasonographer, neurosurgeon, neonatologist, geneticist, and social worker is necessary to provide integrated care. In addition, full parental disclosure of currently known ben-

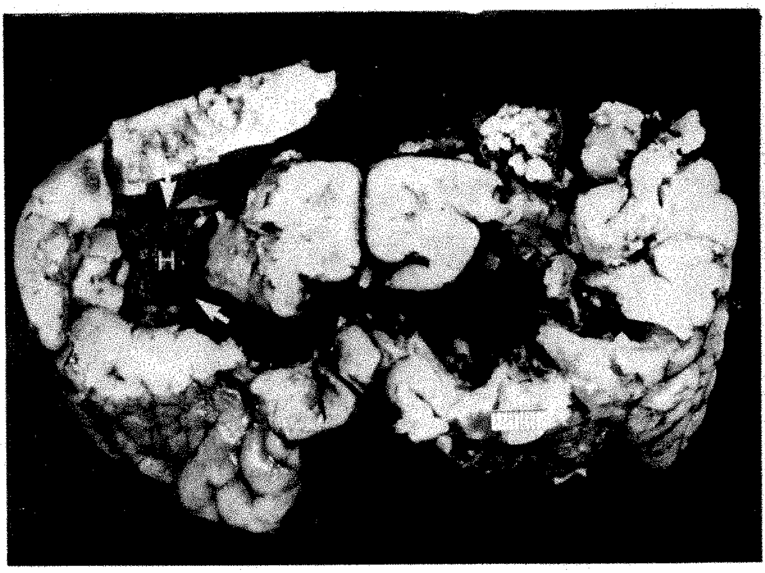

Fig. 4B. Section of brain demonstrating intraventricular hemorrhage *(H)* resulting from cephalocentesis.

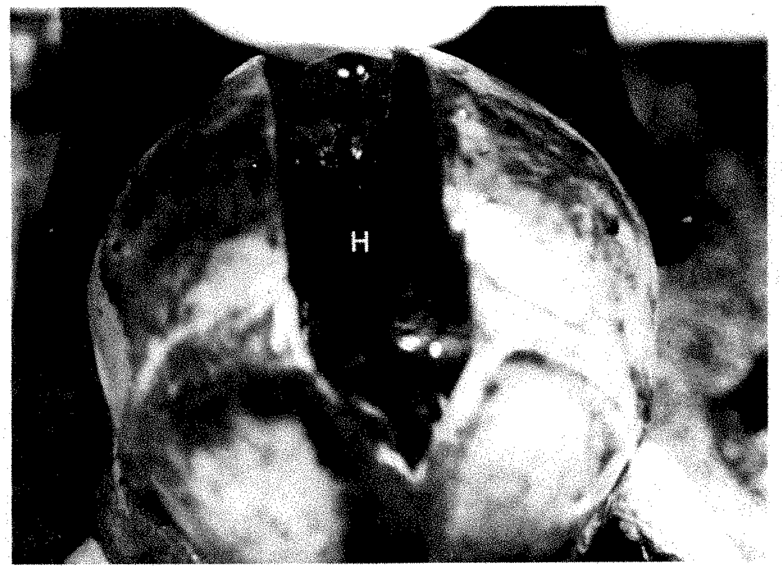

Fig. 4C. Subdural hematoma *(H)* resulting from cephalocentesis.

efits and risks of the procedure is mandatory, so that consent is fully informed concerning this therapeutic intervention of uncertain efficacy.[12]

We recommend delivery of the fetus with hydrocephalus once pulmonary maturity is demonstrated. To facilitate this goal, weekly amniocenteses are performed beginning at 36 weeks of gestation. This recommendation is based upon the potential ill effects of prolonged compression of the cerebral cortex.[11] At the present time, there is no clear guideline when preterm delivery is indicated because of worsening hydrocephalus prior to fetal lung maturity. The risks of respiratory distress syndrome with subsequent delay in neurosurgical ventriculoperitoneal shunt placement must be balanced against the potential ill effects of progressive ventriculomegaly. Serial sonography may be helpful in determining the management decision. If delivery prior to fetal lung maturity is elected, then maternal corticosteroid administration is recommended to reduce the risk occurrence and the severity of respiratory distress syndrome in the neonate.

Cephalocentesis was performed in 11 cases in this series. We feel that the procedure, when indicated, is facilitated by sonographic guidance. Fig. 2 illustrates how an 18-gauge needle can be guided into the fetal skull through the maternal abdomen during local anesthesia. After aspiration of cerebrospinal fluid, overlapping of the cranial sutures can be observed with ultrasound (Fig. 3, *A* and *B*). Throughout the procedure the needle tip can be observed (Fig. 3, *C*).

It is not surprising that perinatal death occurred in 10 of 11 (91%) cases in which cephalocentesis was performed in this series. A recent review of 41 reported cases of cephalocentesis revealed that 23 infants were stillborn and 15 infants died in the neonatal period.[13] The morbid nature of this procedure is also emphasized by the sonographic visualization of intracranial bleeding which was observed in three of our cases (Fig. 3, *D*) and the pathologic confirmation of intracranial hemorrhage after cephalocentesis in seven cases (Figs. 4A, 4B, and 4C). It is acknowledged that some of the perinatal morbidity and mortality reported in association with cephalocentesis may have been due to the fact that the primary intent of the operator was to diminish maternal morbidity or to detect associated anomalies which were not compatible with postnatal life.

We believe that in view of the potential for normal, and even superior, intelligence for those infants with hydrocephalus given optimum neonatal neurosurgical care,[7-10] fetuses with isolated hydrocephalus and macrocephaly are best delivered by cesarean section. In those instances, however, where hydrocephalus is associated with anomalies suggestive of very poor prognosis (for instance, alobar holoprosencephaly,[14] thanatophoric dysplasia with cloverleaf skull,[15] or craniofacial duplication[16]), cephalocentesis and subsequent vaginal delivery may be the most appropriate form of management. Because cortical mantle thickness correlates poorly with subsequent intelligence,[4] we do not advocate cephalocentesis in cases of severe isolated hydrocephalus. If there is no demonstrable cortex, however, then hydranencephaly is most probably present; since this condition has a dismal prognosis, cephalocentesis might be appropriate.

Seven infants in our series were delivered vaginally without cephalocentesis. One neonate in this group had a vaginal delivery after a successful induction of labor and uneventful intrapartum course. In the absence of documented ill effects of vaginal delivery for the hydrocephalic fetus when cephalocentesis is not performed, we believe that cesarean section may not be necessary for all cases if there is normal progress in labor. Attempted vaginal delivery may be appropriate when the fetus in a vertex presentation has normal-sized head (head biometry <2SD above the mean for gestational age[17]) or has mild macrocephaly (for instance, biparietal diameter of 98 mm at 38 weeks of gestation). When fetal hydrocephalus is associated with a meningomyelocele, however, cesarean section may be less traumatic than vaginal delivery.[18]

In conclusion, we have discussed current approaches to the management of fetal hydrocephalus based on our experience with 53 consecutive cases. A uniform plan of management is not possible for fetal hydrocephalus, since the etiologies of this condition are many and widely varied. Determination of any abnormalities associated with hydrocephalus is essential for optimal obstetric care, as management decisions may be greatly influenced by the presence and nature of these abnormalities. Although our ability to detect associated abnormalities has improved, it continues to be far from perfect. With advances in antenatal diagnosis, this accuracy will hopefully improve. Even with accurate antenatal diagnosis, however, therapeutic uncertainties may remain. Among these are the place of elective delivery prior to fetal lung maturity when there is rapid and progressive increase in the lateral ventricle–hemispheric width ratio, the role of ventriculoamniotic shunt placement and the delineation of appropriate candidates for this procedure, the possibility of transabdominal cephalocentesis with removal of a limited amount of cerebrospinal fluid prior to delivery without worsening fetal prognosis, and the safety of vaginal delivery for the infant with hydrocephalus in vertex presentation when there is normal progress of labor.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ALLAN B. WEINGOLD, Washington, D. C. Dr. Chervenak and his colleagues have addressed a clinical problem intense with ethical overtones and have done so with sensitivity and objectivity. Viewed from the perspective of the developing field of fetal therapy, this paper must be considered as a fairly strong negative statement, at least with reference to active in utero intervention.

I want to reemphasize some critical points brought out in this manuscript. First, isolated hydrocephalus is a relatively uncommon event. Ten percent of the patients reviewed had chromosomal abnormalities; 73% had other abnormalities involving virtually every organ system including one systemic infection with toxoplasmosis. The most common associated anomaly was spina bifida occurring in one third of the patients, and other significant intracranial anomalies occur frequently. Of particular concern is the fact that in 26% of the patients the diagnosis of the associated anomaly was not made during the antepartum period. In more recent cases, however, this false negative percentage was lower.

Second, the mean gestational age at diagnosis was 28 weeks even in the most recent series. Given the fact that intervention, of any kind, should be based on serial sonographic evidence of progressive ventricular distention, can we realistically expect significant improvement in outcome unless earlier diagnosis and intervention can be obtained? Dr. Chervenak, how early in pregnancy do you feel that we can, with current technology, make a diagnosis of hydrocephalus with accuracy? Most importantly, what is the false positive rate at 20 weeks of pregnancy when the lateral ventricle–to–hemispheric width ratio is usually in the 50% range? How early will we realistically be able to intervene therapeutically if ventriculo–amniotic fluid shunting proves to be feasible and safe? Bearing in mind your data on the high incidence of intracranial bleeding following cephalocentesis, does it not seem likely that early intervention would be associated with a higher risk of shunt procedure complications?

Third, turning to the management of the cases diagnosed in the third trimester of pregnancy, it is important to reemphasize the point made by McCullough and Balzar-Martin[1] that cortical mantle thickness correlates poorly with subsequent intelligence, particularly in the isolated cases where in vitro therapy may be most applicable.

It is clear that cesarean section provides a relatively atraumatic mode of delivery for the fetus. Our own experience strongly substantiates the need for vertical incisions when macrocephaly exists. This has considerable negative implications for the mother. What, then, were your criteria for vaginal delivery versus elective cesarean section? Were these decisions made jointly with the patient and to what degree did the hope for fetal survival influence that decision? If the direction of management is for survival with the best potential outcome, we have operated on the premise that progressive ventriculomegaly should be managed by delivery after 32 weeks following corticosteroid administration.

Finally, to close on a more optimistic note, let me stress a point made in the article. We anticipate that with further technologic developments our ability to discriminate isolated fetal hydrocephalus will improve. Further efforts at antenatal diagnosis should endeavor to identify all abnormalities in association with fetal hydocephalus as even minor structural abnormalities may be markers of a brain dysgenesis more severe than isolated ventriculomegaly. A technique of current potential is in vitro computed tomographic scanning and, on the near horizon, is nuclear magnetic resonance.

We look forward with great anticipation to the continued contributions of Dr. Chervenak and co-workers who have already added so much to our understanding and facility in antenatal diagnosis.

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DR. JOHN PATRICK, London, Ontario, Canada. I wonder if it is time to concern ourselves with how this sort of diagnostic technique, in this case, of the brain, affects our relation with patients during the last trimester of pregnancy. I want to ask our presenter today if he has any special management suggestions for what we do after he and his colleagues make a diagnosis like this in the management of the woman for the rest of pregnancy, because it has been my experience that it is incredibly difficult.

DR. FEDERICO MARIONA, Detroit, Michigan. With the increase in the use of ultrasound in areas that are really not ready for Level II, how many patients were you seeing from outside sources with fetuses that were, in fact, not hydrocephalic at the time that you studied them? Also, with the frequent appearance of hydramnios in women who have a hydrocephalic fetus and in the case of a hydrocephalic fetus that is swallowing very poorly, would you use a tocolytic agent if the patient goes into labor prematurely?

DR. ROBERT C. GOODLIN, Omaha, Nebraska. We have delivered five hydrocephalic fetuses with very large heads who had no other anomalies and the outcome has been very favorable. Although cephalic fetuses may not "fit together" once the surgeon drains the head, I have the feeling that we should continue to do this. What is the outcome of very large hydrocephalic infants without other anomalies? Do the authors have data on this?

Dr. Chervenak (Closing). Recently, we reported a series of 13 cases of fetal hydrocephalus diagnosed before 24 weeks of gestation[1]; there were no diagnostic errors. It is of note, however, that the lateral ventricle–to–hemispheric width ratios were initially normal in two cases, indicating that serial sonographic examinations may sometimes be necessary to make the diagnosis. In addition, as the natural history of ventricular enlargement early in gestation is uncertain, the diagnosis of ventriculomegaly is most secure if progressive ventricular enlargement is documented with serial sonography. Nonetheless, our experience suggests that it is possible in some cases to diagnose fetal hydrocephalus early enough in gestation to provide the option of termination of pregnancy.

I believe that the therapeutic benefit of ventriculoamniotic shunt placement remains uncertain. Experience in humans[2] has not been as encouraging as it was in monkeys.[3] At this time, however, it is not appropriate to make a definitive statement that shunt placement is never indicated.

In my view, ethical analysis provides greater understanding into the dilemma of whether to perform cesarean section or cephalocentesis when hydrocephalus occurs with macrocephaly.[4] With isolated hydrocephalus, cesarean section is recommended because of substantial beneficence-based obligations to the fetus. These obligations are based upon the potentially destructive nature of cephalocentesis and the possibility for normal, even superior, intelligence in this group. If, however, the mother refuses cesarean section, cephalocentesis would be performed because beneficence-based obligations to the mother assume greater weight, as there is substantial risk of maternal death if labor is allowed to continue without decompression of the enlarged fetal head. If hydrocephalus is associated with severe anomalies, such as cloverleaf skull, alobar holoprosencephaly, or craniofacial duplication, cephalocentesis is recommended to avoid the maternal ill effects of cesarean section, as beneficence-based obligations to the fetus are not as substantial (Chervenak FA, McCullough L. An ethical analysis of the intrapartum management of hydrocephalus associated with macrocephaly. Submitted for publication).

Dr. Patrick makes an important point. Our experience has shown that contact with a perinatal social worker, as well as antepartum introduction to the neurosurgeon and neonatologist, are invaluable in providing support to the patient.

Concerning Dr. Mariona's comments, errors in the diagnosis of hydrocephalus occur in approximately one third of cases prior to the examination of a patient in a referral center. This emphasizes the need for a careful confirmation of fetal hydrocephalus prior to obstetric intervention. We use tocolysis when necessary to arrest premature labor in a pregnancy complicated by fetal hydrocephalus.

In reference to Dr. Goodlin's comment about the neonatal outcome of isolated hydrocephalus associated with macrocephaly, we do not have sufficient clinical data to address this issue meaningfully. Our one survivor with isolated hydrocephalus is currently doing well with an Intelligence Quotient of 98. Lorber's[5] study of a larger number of neonates with severe isolated hydrocephalus demonstrates that the outcome is often good for this subgroup.

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β-Adrenergic–receptor and adenylate cyclase properties in pregnant and nonpregnant guinea pig myometrium

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Stimulation of myometrial β-adrenergic receptors by agonists results in myometrial relaxation through adenylate cyclase–dependent mechanisms. Their activity is modulated by local or systemic estrogen-progesterone milieu. During pregnancy, guinea pigs demonstrate an ovarioplacental shift for progesterone production at ≤0.35 gestation and a gradual increase in estrogen levels. To investigate whether these changes affect myometrial β-adrenergic–receptor and adenylate cyclase properties, we directly characterized β-adrenergic receptors and adenylate cyclase in myometrial plasma membranes from time-dated pregnant (term 65 days), postpartum, and nonpregnant virgin guinea pigs by well-established in vitro methods. In all animals, there appeared to be a high-affinity, low-capacity, single class of binding sites with pharmacologic specificities consistent with the β_2-adrenergic–receptor subtype. The dissociation constants of these β-adrenergic receptors were significantly decreased in pregnant animals at approximately ≥0.80 gestation when compared to pregnant animals at 0.35 to 0.40 gestation, nonpregnant animals, and postpartum animals. Moreover, the total number of β-adrenergic receptors was significantly higher at ≥0.80 gestation than in the other groups. Basal adenylate cyclase activity increased in animals from 0.35 to ≥0.8 gestation and was higher than that of postpartum animals and nonpregnant animals. The degree of adenylate cyclase stimulation by (−)isoproterenol was higher at term than at an earlier point in gestation or in nonpregnant and postpartum animals. These results indicate that, with advancing gestation in pregnant animals, there is an up-regulation of β-adrenergic–receptor and adenylate cyclase function. (AM J OBSTET GYNECOL 1985;151:943-50.)

Key words: β-Adrenergic receptors, adenylate cyclase, myometrium, pregnancy

Uterine contractility is influenced by the relative densities of α- and β-adrenergic receptors.[1] Stimulation of β-adrenergic receptors in human or animal myometrium by β-adrenergic–receptor agonists results in smooth muscle relaxation. This is most likely mediated through an adenylate cyclase–dependent mechanism.

It appears that myometrial β-adrenergic–receptor properties are partly regulated by local or systemic estrogen-progesterone levels as well as presynaptic adrenergic input. Although β-adrenergic–receptor activity in myometrium from hormonally treated, nonpregnant animals has been studied,[2-4] similar experiments in pregnant animals which show marked in vivo estrogen-progesterone changes with advancing gestation have not been reported in detail.[5] Furthermore, the fact that pregnancy, in some species, may induce a decrease in presynaptic adrenergic input to myometrium, presumably because of local progesterone production,[6-8] makes the pregnant animal an attractive model to study the in vivo regulation of β-adrenergic–receptor properties.

These observations raise the question whether local hormonal production and functional denervation result in up-regulation of β-adrenergic–receptor density and adenylate cyclase activity. As a first step to answering this question, we have measured β-adrenergic–receptor properties and adenylate cyclase activity in nonpregnant (nulligravid), pregnant, and postpartum (primiparous) guinea pigs. This species was chosen for two reasons. First, in contrast to previously studied pregnant rabbits,[5] it demonstrates the aforementioned changes in local progesterone production (ovarioplacental shift) and sympathetic denervation with pregnancy.[6-8] Second, pregnant guinea pigs bear significant hormonal similarities to the human. Thus, a study of β-adrenergic–receptor function in guinea pig myometrium may provide important information on human myometrial physiologic characteristics.

Material and methods

Materials. The following drugs were donated by the companies indicated: (±)isoproterenol, (±)norepinephrine, and (±)epinephrine, Sterling-Winthrop; (±)

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propranolol, Ayerst. Other drugs and materials were obtained from commercial sources and were of reagent grade. $(\pm)^{125}$I-hydroxybenzylpindolol, a nonspecific β-antagonist, was obtained from New England Nuclear on a monthly basis, stored at −20° C, and diluted in drug buffer immediately before use (2.2 Ci/μmol). α-^{32}P-adenosine triphosphate (20 to 40 Ci/mmol) was obtained from New England Nuclear and [3′,8′-^{3}H], 3′5′-cAMP (15 Ci/mmol) from ICN.

Animals. Virgin female guinea pigs weighing 400 to 600 gm and primigravid guinea pigs with time-dated gestations and weighing 600 to 1200 gm were obtained from Perfection Breeders. Prior to sacrifice, they were housed in our animal care facility for 2 to 20 days. Some pregnant guinea pigs were allowed to deliver and were put to death in the postpartum period (1 to 5 days).

Animals were anesthetized with 40 to 55 mg/kg of sodium pentobarbital intraperitoneally. At laparotomy, a hysterectomy was performed. Attached adipose and vascular tissue was removed from the uterus and the cervical region was excised. Hysterotomy was performed in pregnant animals and the products of conception were removed from the endometrial cavity. Tissue was snap-frozen in liquid nitrogen and stored at −70° C until used.

Tissue preparation. Uterine tissue was thawed in ice-cold buffer A containing 0.005 mol/L of ethylenediaminetetraacetic acid (EDTA), 0.025 mol/L of sucrose, 0.9% sodium chloride, and 0.02 mol/L of Tris hydrochloride, buffer, pH 7.5. The thawed uterus was then split along each horn and the endometrium was scraped. The uterus was placed in 5 to 10 volumes per gram of wet weight of buffer A, minced finely with scissors, and sonicated on a Brinkman Polytron (setting 7, twice, for 30 seconds each time). The homogenate was then filtered through eight layers of cheesecloth.

To an aliquot of tissue that was to be used for β-adrenergic–receptor assay, an equal volume of 1.2 mol/L of potassium chloride was added. This mixture was then incubated on ice with constant stirring for 10 minutes and subsequently centrifuged at 80,000 × g (15 minutes at 4° C). The pellet was resuspended in ice-cold buffer B (0.9% sodium chloride and 0.02 mol/L of Tris hydrochloride, pH 7.5) and was then centrifuged as before. The final pellet was resuspended in buffer B (100 to 300 volumes per gram of wet weight) and was used for β-adrenergic–receptor experiments.

An aliquot of the original homogenate to be used for adenylate cyclase experiments was centrifuged at 80,000 × g for 15 minutes (4° C) and the pellet resuspended in 2 to 4 ml of ice-cold buffer B. It was preincubated for 10 minutes at 4° C in the presence of 0.5 mmol/L of dithiothreitol, 0.17 mmol/L of ethyleneglycol-*bis*(β-amino-ethyl ether)N,N′-tetraacetic acid (EGTA), 1 mmol/L of hydrochloric acid, 0.2% bovine serum albumin, and 5 mmol/L of magnesium chloride, pH 7.45. The suspension was then recentrifuged as previously outlined. The resulting pellets were resuspended in ice-cold buffer B (100 to 150 volumes per gram of wet weight) and used in the assay.

Binding studies. For all binding studies, an aliquot (0.15 ml, 5 to 45 μg of protein) of crude membrane preparation was incubated with $(\pm)^{125}$I-labeled hydroxybenzylpindolol and appropriate drugs in a total volume of 0.25 ml (0.9% sodium chloride, 20 mmol/L of Tris hydrochloride, 0.17 mmol/L of EDTA, 1 mmol/L of hydrochloric acid, and 0.2% bovine serum albumin, pH 7.45).

To determine the concentration of binding sites and their affinity to $(\pm)^{125}$I-labeled hydroxybenzylpindolol, samples were incubated for 45 minutes at 37° C, in triplicate. The amount of specifically bound $(\pm)^{125}$I-labeled hydroxybenzylpindolol was determined at five to eight concentrations of $(\pm)^{125}$I-labeled hydroxybenzylpindolol (15 to 400 pmol/L). The data were analyzed by the method of Scatchard[9] to provide estimates of the maximum density (B_{max}) of β-adrenergic receptors, femtomoles per milligram of protein per uterus and the dissociation constant (K_D) of $(\pm)^{125}$I-labeled hydroxybenzylpindolol, picomoles per liter.

To determine the potency of the various drugs in inhibiting $(\pm)^{125}$I-labeled hydroxybenzylpindolol binding, samples were incubated for 45 minutes at 37° C, in duplicate, with various concentrations of each drug. $(\pm)^{125}$I-labeled hydroxybenzylpindolol was present at concentrations of 17 to 25 pmol/L. Inhibition constants (K_I) were calculated by the logit-log transformation of data and application of the Cheng and Prussoff[10] correction: $K_I = IC_{50}/(1 + [L]/K_D)$ where IC_{50} is the concentration of competing ligand that inhibits 50% $(\pm)^{125}$I-labeled hydroxybenzylpindolol binding; [L] is the concentration of $(\pm)^{125}$I-labeled hydroxybenzylpindolol in picomoles, and K_D is the dissociation constant of $(\pm)^{125}$I-labeled hydroxybenzylpindolol as determined by Scatchard analyses in picomoles per liter.

Reactions were stopped by adding 5 ml of buffer B to each tube; the samples were then rapidly filtered through Whatman GF/C glass fiber filters. Each filter was washed with an additional 15 ml of buffer B and dried. Radioactivity was determined in a γ-counter (Packard Autogamma 500C, 3-inch Na(TL) crystal, efficiency 70% to 79%).

Specific binding of $(\pm)^{125}$I-labeled hydroxybenzylpindolol was defined as the amount bound in the absence of competing ligand minus the amount bound in the presence of 10 μmol/L of (±)propranolol. Specific binding routinely represented 65% to 80% of total $(\pm)^{125}$I-labeled hydroxybenzylpindolol binding.

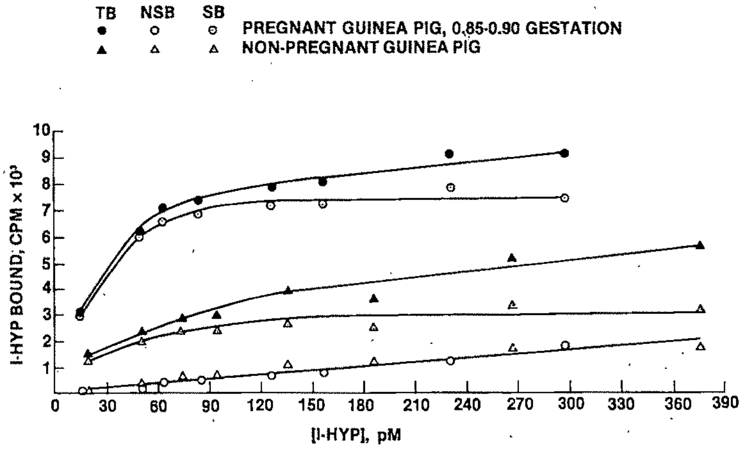

Fig. 1. Binding assay of (±)^{125}I-labeled hydroxybenzylpindolol *(I-HYP)*. To determine the density of binding sites and K_D, the amount of specific *I-HYP* binding was determined at various concentrations of *I-HYP*. Data are in counts per minute of two representative equilibrium experiments: *(A)* nonpregnant animal and *(B)* pregnant animal (55 to 65 days' gestation). *TB:* Total binding; *NSB:* nonspecific binding (10^{-5} mol/L of (±)propranolol); *SB:* specific binding.

Table I. β-Adrenergic receptors in guinea pig myometrium: Equilibrium characteristics

Group	*n*	K_D **(pmol/L)*	B_{max} *†(fmol/mg protein)*
A: Nonpregnant	7	60.70 ± 8.48	55.45 ± 14.14
B: Early pregnancy (20-25 days' gestation)	5	52.87 ± 12.54	91.36 ± 15.23
C: Late pregnancy (≥55-65 days' gestation)	8	28.06 ± 3.65	2878.87 ± 648.22
D: Post partum (1-3 days)	6	41.47 ± 8.88	712.97 ± 69.62

Results are mean ± SEM.

*$p < 0.05$ when K_D in group C is compared to that of group A, B, or D.

†$p < 0.001$ when B_{max} in group C is compared to that of group A, B, or D. B_{max} in group D is greater than that in group A or B.

Adenylate cyclase assay. Adenylate cyclase activity was determined by measuring the conversion of α-^{32}P–labeled adenosine triphosphate to 3'5'-cyclic-^{32}P-labeled adenosine monophosphate and isolating the product by a modification of a method developed by Salomon et al.[11] The reaction was carried out in triplicate at 30° C for 15 minutes in a final volume of 0.2 ml containing 0.1 ml of membrane fraction (8 to 60 μg of protein per tube) and 50 mmol/L of Tris hydrochloride, pH 7.5, 0.45% sodium chloride, 0.5 mmol/L of adenosine triphosphate, 5 mmol/L of magnesium chloride, 0.17 mmol/L of EGTA, 0.05 mmol/L of cyclic adenosine monophosphate, 0.5 mmol/L of dithiothreitol, 0.75 mmol/L of 3-isobutylmethylxanthine, 0.1 mg/ml of creatine kinase, 10 mmol/L of creatine phosphate, and 1 to 2 × 10^6 cpm of α-^{32}P–labeled adenosine triphosphate. Appropriate drugs were added as required. The reaction was initiated by adding the membrane fraction to the incubation tubes. It was linear with respect to time (up to 20 minutes) and protein concentration. The reaction was terminated by the addition 0.25 ml of a solution containing 50 mmol/L of Tris hydrochloride (pH 7.5), 5 mmol/L of adenosine triphosphate, 1 mmol/L of 3'5'-cyclic adenosine monophosphate, and 5% sodium dodecyl sulfate. Tritium-labeled cyclic adenosine monophosphate (10,000 to 25,000 cpm per tube) was added to all tubes to serve as an internal standard and monitor the efficiency of recovery. The tubes were then placed in boiling water for 10 minutes and then allowed to cool down to room temperature. 3'5'-Cyclic-^{32}P-labeled adenosine monophosphate was isolated with sequential Dowex (AG 50W-X4, 200 to 400 mesh, Bio-Rad) and neutral alumina columns essentially as described by Salomon et al. and counted in a scintillation counter (Packard). Recovery of 3'5'-cyclic adenosine monophosphate was determined by the percentage of recovery of the tritium-labeled 3'5'-cyclic adenosine monophosphate external standard in each sample.

Results have been corrected for the percentage of

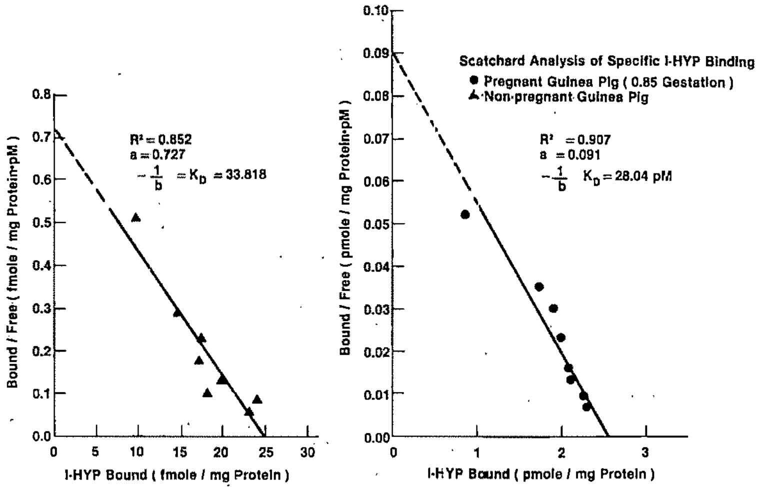

Fig. 2. Scatchard analysis of specific *I-HYP* binding data (from Fig. 1). The slope of the line $(-\frac{1}{b})$ provides an estimate of K_D while the x axis intercept provides a value for the density of receptors (B_{max}).

recovery (routinely >40% to 60%). Enzymatic activity was expressed as picomoles of 3′5′-cyclic adenosine monophosphate produced per milligram of protein per minute per uterus.

Protein assay. Protein concentrations were determine according to the method of Bradford[12] with bovine serum albumin used as a standard.

Statistical analysis. We used analysis of variance to test the null hypotheses that there were no differences in K_D, B_{max}, and K_I and adenylate cyclase activity with advancing gestation. A p value of <0.05 was considered significant.

Results

In preliminary experiments, the kinetic behavior of (±)^{125}I-labeled hydroxybenzylpindolol binding to myometrial membranes was characterized. The binding was rapid, reached equilibrium by 45 minutes, and remained stable for at least 80 minutes. It was reversible by addition of 10 μmol/L of (±)propranolol and reached nonspecific binding levels by 120 minutes (unpublished observations).

The equilibrium characteristics of specific (±)^{125}I-labeled hydroxybenzylpindolol are shown schematically in Figs. 1 and 2 and the overall results are shown in Table I. Fig. 1 demonstrates that specific (±)^{125}I-labeled hydroxybenzylpindolol binding was saturable while total binding was not. The latter increased in parallel with nonspecific binding. Scatchard analysis of specific binding (Fig. 2) revealed that (±)^{125}I-labeled hydroxybenzylpindolol bound to a single class of high-affinity binding sites.

Table I shows that the K_D for (±)^{125}I-labeled hydroxybenzylpindolol binding decreased in late pregnancy and was significantly lower from the values observed in nonpregnant, early pregnant, or postpartum animals. This indicates an increase in the β-adrenergic–receptor affinity for binding (±)^{125}I-labeled hydroxybenzylpindolol in guinea pigs in late gestation. Moreover, the highest B_{max} was observed in pregnant guinea pigs in late gestation, and this value was significantly different from those in the other groups. Finally, B_{max} in postpartum guinea pigs was significantly higher than that in the nonpregnant animals or the animals in the early stages of pregnancy but lower than that in guinea pigs in late pregnancy.

As shown in Fig. 3 and Table II, (±)^{125}I-labeled hydroxybenzylpindolol binds to a class of receptors that demonstrate: (1) pharmacologic specificity consistent with the β_2-subtype of β-adrenergic receptors [(−)isoproterenol > (−)epinephrine, >(−)norepinephrine] and (2) steric specificity in that the (−)isomers are more potent competitive inhibitors than the (+)isomers. Similar steric specificity was seen when (±)propranolol was compared to the (+)propranolol inhibition. There was no significant difference in the K_I in myometrial membranes obtained from animals at different points in their life cycles.

Tables III and IV show the overall results for adenylate cyclase activity. Table III shows that there was a marked increase in basal activity in guinea pigs in late pregnancy. Addition of guanosine triphosphate resulted in a significant rise in basal adenylate cyclase activity in all groups (50% to 100%). However, the highest rise was observed in uteri from guinea pigs in late pregnancy. Similarly, sodium fluoride induced a significant increase in adenylate cyclase activity and appeared to be the most potent stimulator in all groups.

Table II. Effect of β-adrenergic receptor agonists and antagonists on specific $(\pm)^{125}$I-labeled hydroxybenzylpindolol binding to guinea pig myometrial membranes

Drug	*n*	K_I **(μmol/L)*
(−)Isoproterenol	8	0.50 ± 0.10
(+)Isoproterenol	6	47.25 ± 7.64
(−)Epinephrine	8	2.12 ± 0.08
(+)Epinephrine	6	15.69 ± 3.11
(−)Norepinephrine	8	29.12 ± 8.80
(+)Norepinephrine	6	247.07 ± 59.65
(±)Propranolol	4	0.00298 ± 0.00076
(+)Propranolol	7	0.12 ± 0.02

Results are mean ± SEM of four to eight experiments in nonpregnant, pregnant, and postpartum guinea pigs.

*K_I was determined from logit transformation of the data and by applying the correction proposed by Cheng and Prussoff. Competing drugs were evaluated at six or more concentrations.

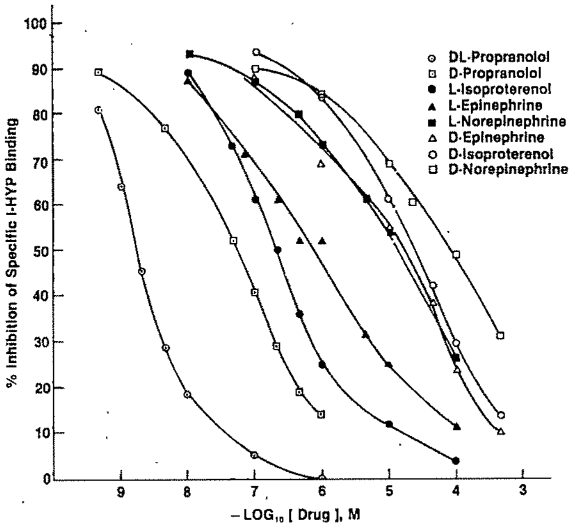

Fig. 3. Competition curves for adrenergic agonists and antagonists. Each point is the mean of four to six experiments from nonpregnant, pregnant, and postpartum animals.

The degree of stimulation by sodium fluoride was similar in all groups and its effects were not dependent on the presence of guanosine triphosphate.

In Table IV, the degree of stimulation by (−)isoproterenol is shown. (−)Isoproterenol stimulation was observed only in the presence of guanosine triphosphate (10^{-5} to 10^{-4} mol/L). Uteri from guinea pigs in late pregnancy demonstrated a significantly higher percentage of change (95% to 100%) over basal adenylate cyclase activity in the presence of guanosine triphosphate than that observed in the other groups (29% to 58%). Addition of (±)propranolol inhibited adenylate cyclase–stimulated activity by (−)isoproterenol down to basal levels.

Comment

In these experiments, we studied the properties of β-adrenergic–receptors and the activity of adenylate cyclase in pregnant and nonpregnant guinea pig myometrium. We observed significant changes in β-adrenergic–receptor function in late gestation.

In all groups, $(\pm)^{125}$I-labeled hydroxybenzylpindolol bound specifically to one class of saturable binding sites. Their pharmacologic specificity, as seen in our competition studies, suggests that these sites are primarily the β_2-subtype of β-adrenergic receptors.

Binding characteristics of these receptors in guinea pig myometrium are different in pregnant and nonpregnant animals. First, there was a decrease in the value of K_D for $(\pm)^{125}$I-labeled hydroxybenzylpindolol in late pregnancy when compared to the value in nonpregnant animals, early pregnancy, and postpartum guinea pigs. In contrast, the K_I for several β-agonists and antagonists did not change, when studied at different points of the animals' life cycles. Second, there was a highly significant and remarkable increase in the density of β-adrenergic receptors in late pregnancy with a gradual decline in the early postpartum period.

Furthermore, the observations concerning adenylate cyclase activity are in agreement with our β-adrenergic–receptor data in that both basal activity and the degree of stimulation by (−)isoproterenol are higher in animals closer to term than at any of the other life points studied. In contrast, sodium fluoride stimulation, which does not depend primarily on β-adrenergic–receptor function, was equivalent in all animals.

The etiology of this up-regulation of β-adrenergic–receptor density and function in the guinea pig in late pregnancy is not known. It is unlikely that this reflects an increase in smooth muscle cell number and it might be secondary to increased β-adrenergic–receptor and adenylate cyclase protein synthesis or uncovering of previously spare binding sites.

There are at least two potential explanations for the observed changes. First, from work in nonpregnant rats, rabbits, and sheep,[2-4] myometrial β-adrenergic–receptor density increased as a result of exogenous administration of estradiol. However, β-adrenergic–receptor K_D did not show any significant changes. Since estrogen production increases with advancing gestation in guinea pigs,[13] it is conceivable that part of the up-regulation of β-adrenergic–receptor function is due to that change.

Another possibility needs to be considered. The pregnant guinea pig is unique in that, similar to the situation in human pregnancy, there is local placental progesterone production after 25 to 35 days' gestation (ovarioplacental shift).[14] This is thought to be important for pregnancy maintenance and uterine relaxation. Local progesterone secretion results in a decrease in nor-

Table III. Adenylate cyclase activity in guinea pig myometrial membranes (3′5′-cyclic adenosine monophosphate produced in picomoles per milligram of protein per minute)

Group	*n*	*Basal**	*Basal plus GTP (10^{-4} mol/L)*†*	*Basal plus GTP (10^{-4} mol/L) plus NaF (10^{-2} mol/L)*‡*
A: Nonpregnant	4	117.19 ± 31.89	185.58 ± 35.87	1121.41 ± 215.80
B: Early pregnancy (20-25 days' gestation)	3	138.82 ± 24.56	223.99 ± 47.80	1153.47 ± 289.86
C: Late pregnancy (55-65 days' gestation)	3	612.49 ± 49.62	2313.18 ± 347.42	10,568 ± 750.03
D: Post partum (1-3 days)	4	107.24 ± 37.35	208.45 ± 61.09	1700.34 ± 611.42

Results are mean ± SEM. GTP = Guanosine triphosphate; NaF = sodium fluoride.

*$p < 0.01$ for basal, basal plus GTP, and NaF plus GTP adenylate cyclase activity between group C and groups A, B, and D.

†$p < 0.05$, percentage of stimulation by GTP over basal enzyme activity, between group C and groups A, B, and D.

‡Percentage of stimulation by NaF over basal plus GTP activity did not differ between groups.

Table IV. Adenylate cyclase activity in guinea pig myometrial membranes (3′5′ cyclic adenosine monophosphate produced in picomoles per milligram of protein per minute)

Group	*n*	*Basal plus GTP (10^{-4} mol/L)**	*Basal plus GTP (10^{-4} mol/L) plus (−)isoproterenol (10^{-4} mol/L)*†*	*Basal plus GTP (10^{-4} mol/L) plus (−)isoproterenol (10^{-4} mol/L) plus (−)propranolol (10^{-5} mol/L)*
A: Nonpregnant	4	185.58 ± 35.87	241.04 ± 47.63	175.20 ± 41.89
B: Early pregnancy (20-25 days' gestation)	3	223.99 ± 47.80	354.82 ± 78.09	255.60 ± 56.03
C: Late pregnancy (55-65 days' gestation)	3	2313.18 ± 347.42	4301.37 ± 603.76	2715.23 ± 426.30
D: Post partum (1-3 days)	4	208.45 ± 61.09	306.50 ± 104.55	229.35 ± 77.26

Results are mean ± SEM.

*$p < 0.01$ for basal plus GTP and (−)isoproterenol adenylate cyclase activity between group C and groups A, B, and D.

†$p < 0.05$ for percentage of stimulation over basal plus GTP activity by (−)isoproterenol between group C and groups A, B, and D.

epinephrine uptake as well as a decrease in fluorescent adrenergic nerve terminals with pregnancy. This is supported by the observation that similar changes are seen after progesterone pellet implantation in the uterine cavity of nonpregnant, castrated animals.[6-8] Thus functional adrenergic denervation of myometrium during pregnancy may also contribute to the observed up-regulation of β-adrenergic–receptor function. In this context, it is important to note that in pregnant rabbits, a species that does not show an ovarioplacental shift, myometrial β-adrenergic–receptor density decreased and β-adrenergic–receptor K_D remained unchanged with advancing gestation.[5]

The implications of these findings with respect to myometrial contractility would seem to be that, with advancing pregnancy, an increase in the population of β-adrenergic–receptor and adenylate cyclase activity contributes to myometrial relaxation and pregnancy maintenance. Further studies to investigate the apparent changes in α-adrenergic–receptor population in this species as well as the in vitro myometrial contractility would need to be performed to elucidate fully the functional implications of the in vitro β-adrenergic–receptor results.

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Discussion

Dr. James C. Warren, St. Louis, Missouri. Dr. Hatjis would have us believe that in the guinea pig advancing gestation is associated with an increase in the density of myometrial β-adrenergic receptors (β_2-subtype), an increase in basal adenylate cyclase activity, and an increase in its stimulation by (−)isoproterenol in vitro. Overall, this constitutes an up-regulation of β-adrenergic receptors and receptor-dependent adenyl cyclase. There are more receptors present in late gestation (55 to 65 days) than in early pregnancy (20 to 25 days) or nonpregnant and postpartum (1 to 3 days) states, and receptor occupation by (−)isoproterenol induces more adenyl cyclase activity. Such a situation would imply that the uterus is in a state of relative relaxation.

His methodology is "state of the art." He has evaluated receptors with the classically used ^{125}I-labeled hydroxybenzylpindolol, used accepted techniques to account for nonspecific binding, and shown by Scatchard plots and careful ordering of the commonly used inhibitors of specific hydroxybenzylpindolol binding that the receptors evaluated are a single class of high-affinity, low-capacity, β_2-subtypes. The tissue used for incubation was prepared by methods that should minimize differential tissue losses (he used the 80,000 × g pellet). He quantitated adenyl cyclase activity as described by Salomon et al. This is benchmark technique. He assayed activity as basal, basal plus guanosine triphosphate, and basal plus guanosine triphosphate plus sodium fluoride categories. His evaluation of in vitro isoproterenol stimulation is convincing as it is ablated by propranolol.

I can find no fault with his strategy or tactics and I, for one, believe him, with perhaps one exception, his report of a qualitative change in the β_2-adrenergic receptor in late pregnancy whereby the K_D changes from approximately 50 to approximately 28 pmol/L. His statistics indicate that this is significant. Considering the vagaries of Scatchard plotting, the fact that considerable error may occur in estimation of the concentrations of free ligand,[1] that these mostly influence K_D (rather than total binding site density), and that K_D changes are rare in the adrenergic receptor literature, I would prefer (at this time) to reserve judgment as to the qualitative change. Remember that when statistics say the odds are 20:1 that something has changed, it also means that there is one chance in 20 that the result is merely attained by circumstance.

There are two questions I would like to ask Dr. Hatjis. The first relates to the ratio of membrane fraction to nonmembrane fraction in the uterine preparation and possible variation between early and late pregnancy. It is clear that if somehow the physical characteristics of the myometrium change so that late in pregnancy there were relatively more milligrams of membrane per unit weight of total preparation, this alone (with data based on units of binding or activity per milligram of total protein) could provide for his observation. Such an event might occur by in vivo loss of nonmembrane protein from or gain of membrane by the uterus as it moves into late gestation. Such possibilities can be ruled out by use of membrane markers which show that membrane fraction remains constant, such as sodium-potassium-adenosine triphosphatase or 5′nucleotidase. Were any such markers used? Indeed, the cyclic adenosine monophosphate assay with sodium fluoride has been used as such a marker, and, at face value, the observations in Table III could be taken to indicate that the sample of late pregnancy is greately enriched with membrane and it is this enrichment (which has occurred by synthesis of more membrane or loss of nonmembrane protein) that is responsible totally or in part for the observed increase in β-adrenergic receptors and adenylate cyclase. So were any other membrane marker techniques used to evaluate that possibility?

The second question related to α-adrenergic receptors in the guinea pig. There appear to be tremendous species differences in terms of the adrenergic receptors and hormonal effects in various animals. Krall et al.[2] demonstrated that estradiol increased β-adrenergic catecholamine–receptor content in the rat uterus. Working with the rabbit, Roberts et al.[3] found that estradiol increases α-adrenergic receptors, while progesterone decreases α-adrenergic receptors in estrogen-primed animals. Neither steroid had an effect on β-adrenergic receptors. In that study, when the animal was injected with estradiol benzoate alone α-receptors were increased and prepared myometrial strips exhibited spontaneous contractile activity which was dramatically increased by the in vitro addition of norepinephrine to a final concentration of 5 μmol/L. When animals that had been treated with both estrogen and progesterone (α-adrenergic receptors were decreased 75% by progesterone and β-adrenergic receptors were unchanged) were studied, norepinephrine reduced spontaneous myometrial activity. This and other works suggest the biologic importance of the ratio of α- to β-receptors. Dr. Hatjis, your work would be greatly extended by the data on α-adrenergic receptors and in vitro myometrial contractility in early and late pregnancy, to which you referred. Do you have any?

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Dr. William J. LeMaire, Miami, Florida. The myometrium is a well-vascularized tissue, and if I understand the methodology correctly, the membrane preparations are obtained from homogenates of whole myometrium. How, then, can you either rule in or rule out the possibility of changes in vascularity during the pregnant, nonpregnant, or postpartum state?

Dr. Hatjis (Closing). I think that the problem of isolating membrane markers is an old one and has not been solved. The reason that the β-adrenergic–receptor system is such a useful model is not only that the density and pharmacologic characteristics of the receptors can be measured but also because the activity of the adenylate cyclase can be quantitated. Thus an in vitro functional assay is achievable. Although I do have concerns about the probable increase in the amount of protein present, I believe that the changes are in the opposite direction. We are going to repeat some of these experiments, however, and measure DNA content or 5'-nucleotidase, which is supposed to be another membrane marker, in order to amplify our results. Nevertheless, we believe that by showing consistent and parallel changes in both the β-adrenergic receptors and adenylate cyclase activity, we have, at least, partially answered your questions about recovery and membrane markers satisfactorily.

The study of α-adrenergic receptors in myometrial membranes is a fascinating story. To date we have encountered significant problems with regard to α_1- and α_2-adrenergic receptors. Detailed studies indicate that we are dealing with multiple subtypes with different kinetic and equilibrium characteristics. Further work is in progress. A tremendous amount of effort on our part will be required to sort out this type of problem.

Recently we have started to analyze our data using not only Scatchard plots but also established computer-assisted nonlinear curve and statistical analysis models. We hope that this approach will add vigorous statistical validity to our results obtained from linear regression analysis with the Scatchard plots.

We have not done any in vitro contractility studies. This is something that we plan to do in the future, but I am not sure exactly which direction that is going to take. I do not think a classical in vitro field simulation or length-tension study would suffice. One might have to use isolated muscle cells and try to do direct intracellular recordings.

With regard to Dr. LeMaire's question about the vascular supply to the uterus and its contribution to these experiments, we recognize the fact that in every tissue we used, be it the heart or the uterus, there are small-caliber blood vessels present ranging from capillaries to arterioles that cannot possibly be completely separated from uterine smooth cells. In our preparation we have obviously eliminated major blood vessels. That is the only procedure that we have done to satisfy the requirement.

It is not feasible to completely separate smooth muscle reticulum from plasma membranes although enriched fractions have been prepared. We have tried to do fractionation studies but have not been impressed with the results or recovery. To try to sort out blood vessels which in our estimation represent only a small fraction of the total protein present is not going to be an easy task.

Application of Doppler echocardiography in the assessment of fetal cardiac disease

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Pulsed Doppler echocardiography was used in conjunction with real-time two-dimensional and M-mode ultrasound techniques in 19 fetuses between 28 and 40 weeks of gestation. Of these, nine also had echocardiographic examination performed neonatally. In one fetus with complete heart block, pulsed Doppler echocardiography showed tricuspid regurgitation. The ventricular and atrial rates were accurately determined by M-mode ultrasound; pericardial effusion was noted in the two-dimensional examination. The left ventricular stroke volume, as measured by the Doppler technique, was 13.5 ml, and the minute output was 486 ml/min. In another fetus, Doppler studies assisted two-dimensional echocardiography in detecting pulmonary aneurysm. Neonatal echocardiography confirmed this and also showed the presence of tetralogy of Fallot. In three fetuses with ventricular ectopy, 30% to 50% reduction in the right ventricular stroke volume was recorded by the Doppler technique. This study demonstrates the clinical potential of pulsed Doppler echocardiography in assessing fetal cardiac disorders and justifies further investigation to define its clinical utility. (AM J OBSTET GYNECOL 1985;151:951-7.)

Key words: Doppler echocardiography, fetal cardiac disease, congenital heart disease

Recent experience with two-dimensional and two-dimensional–directed M-mode echocardiography has demonstrated the technique's potential in the prenatal investigation of fetal cardiac spatial anatomy and fetal cardiac rhythm disturbances.[1-3] Consequently it is recognized that this capability of noninvasively assessing fetal cardiac disorders can aid prenatal counselling, appropriate management of pregnancy and delivery, and planning of specialized neonatal care. It has been shown in pediatric and adult cardiologic practice that range-gated pulsed Doppler studies can substantially enhance the diagnostic reliability of two-dimensional and M-mode techniques.[4-6] Recently the feasibility of using pulsed Doppler echocardiography in the human fetus has been reported and the technique for assessing intracardiac circulatory hemodynamics and ventricular

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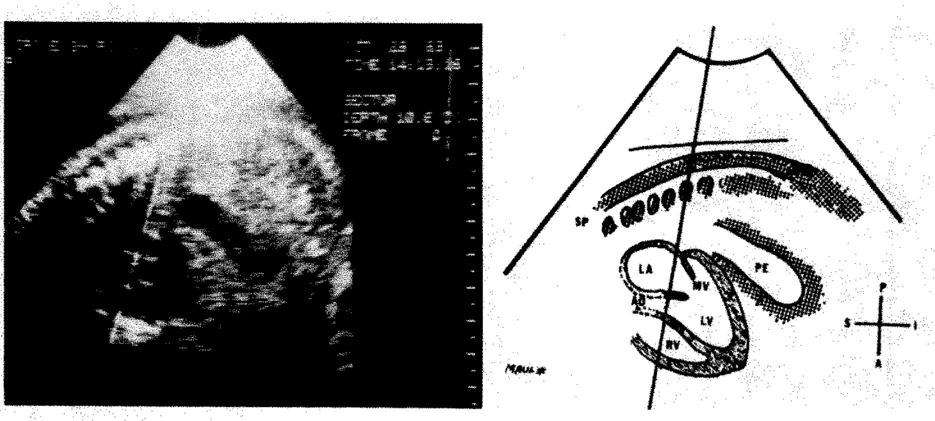

Fig. 1A. Echocardiographic determination of atrial and ventricular rates in a fetus with complete heart block and heart failure. The M-line cursor is passed through the left atrial and right ventricular walls imaged by the two-dimensional echocardiographic technique. *AO*, Aorta; *LA*, left atrium; *LV*, left ventricle; *MV*, mitral valve; *PE*, pericardial effusion; *RV*, right ventricle; *SP*, fetal spine; *VS*, interventricular septum. Spatial orientation: *A*, anterior; *I*, inferior; *P*, posterior; *S*, superior.

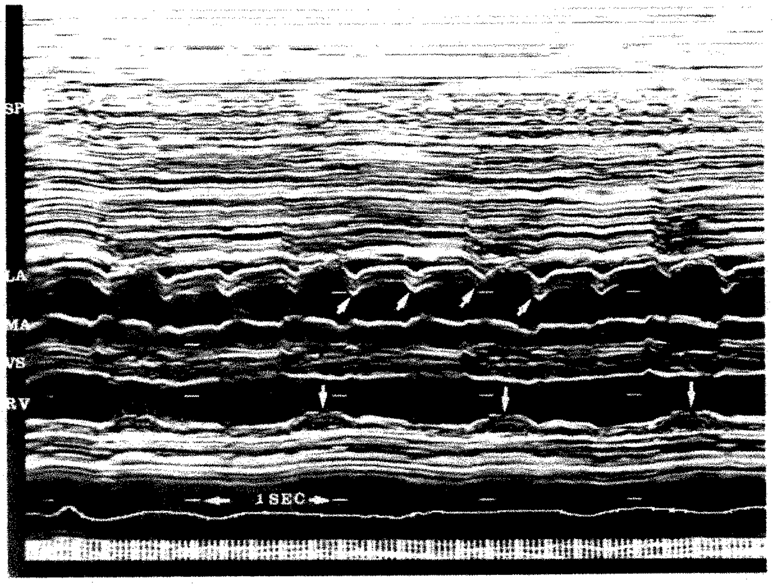

Fig. 1B. Echocardiographic determination of atrial and ventricular rates in a fetus with complete heart block and heart failure. The M-mode echocardiogram showed a relatively rapid left atrial rate of approximately 120 bpm and a ventricular rate of only 50 bpm. The *oblique arrows* show the contractions of the left atrial wall, and the *vertical* arrows denote systolic inward motion of the right ventricular wall. *LA*, Left atrium; *MA*, mitral annulus; *RV*, right ventricle; *SP*, fetal spine; *VS*, interventricular septum.

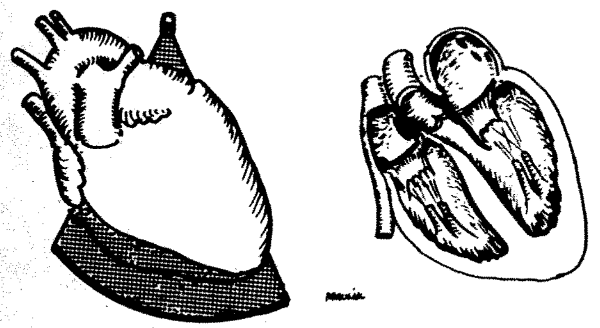

Fig. 1C. Echocardiographic determination of atrial and ventricular rates in a fetus with complete heart block and heart failure. Schematic diagram shows the position of the two-dimensional echocardiographic plane.

output has been described by us.[7] This paper presents our further experience in extending the use of pulsed Doppler ultrasound to complement two-dimensional and M-mode echocardiography for assessing fetal cardiac disorders.

Material and methods

Two-dimensional, M-mode, and pulsed Doppler echocardiographic examinations were performed in 19 fetuses between 28 to 40 weeks of pregnancy. Nine of these fetuses, who also received neonatal echocardiographic evaluation for the verification of prenatal echocardiography findings, constituted the study population of this report. All mothers were appropriately counselled, and informed consents were obtained.

Table I. Fetal echocardiographic diagnoses in the study population

Diagnosis	*No. of cases*
Congenital complete heart block with congestive heart failure*†	1
Tetralogy of Fallot† with pulmonary artery aneurysm*†	1
Ventricular ectopy*†	3
Normal cardiac diagnosis*†	4

*Prenatal echocardiographic diagnosis.
†Neonatal echocardiographic diagnosis.

A commercially available echocardiographic instrument with a 3 MHz transducer was used (Mark VI duplex system, Advanced Technology Laboratories). This offered the modalities of two-dimensional, M-mode, and bidirectional range-gated Doppler echocardiography combined in a single transducer assembly. Permanent records of the Doppler tracings were made on half-inch video cassettes by means of a Panasonic video recorder (Model 8200). Hard copies were also obtained in a paper recorder that constituted an integral part of the echocardiography instrument. Acoustic power output of the instrument in the Doppler mode was kept at the minimum setting (−9 db).

The procedure for Doppler echocardiography in the human fetus has been described in detail by us elsewhere.[7] Briefly, a preliminary two-dimensional real-time ultrasound scan was performed in order to determine fetal orientation in utero and to locate the fetal heart. With appropriate transducer manipulation, var-

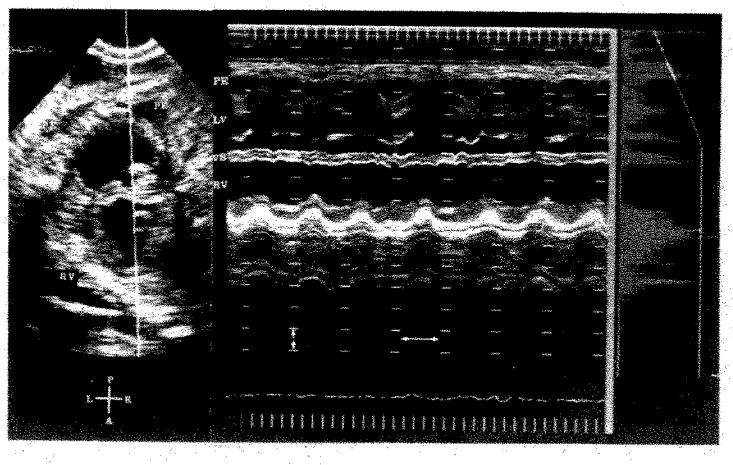

Fig. 2. Doppler examination in a fetus with complete heart block and congestive heart failure. The two-dimensional examination *(left)* showed pericardial effusion spaces *(PE)* behind the left ventricle *(LV)* imaged in the short axis. Spatial orientation is shown at *bottom left* (*A*, anterior; *P*, posterior; *L*, left; *R*, right). The M-mode tracing *(right)* obtained by passing a cursor through the two-dimensional image revealed a ventricular rate of approximately 60 bpm. The *vertical arrows* denote 1 cm distance whereas the *horizontal arrows* denote a 1-second time lapse. *RV*, Right ventricle; *VS*, interventricular septum.

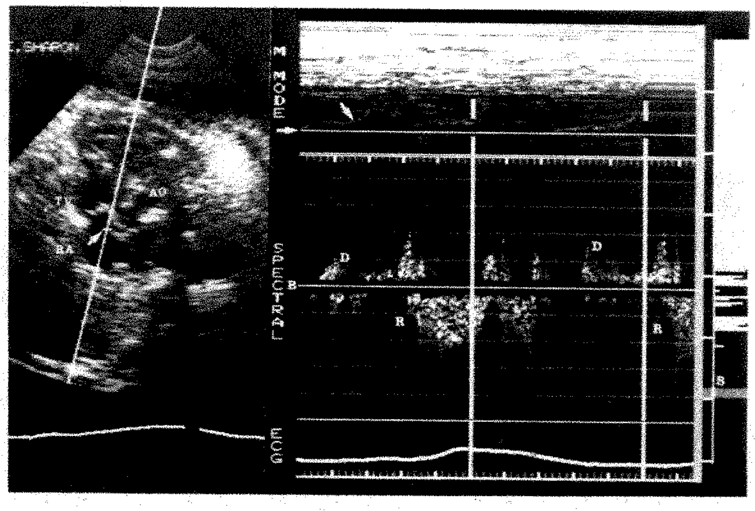

Fig. 3. Doppler identification of tricuspid regurgitation in a fetus with complete heart block and heart failure. The Doppler sample volume *(oblique arrow)* was placed in the right atrium *(RA)* imaged in the aortic *(AO)* short-axis plane *(left)*. The Doppler tracing *(right)* showed prominent reversed flow *(R)* during systole indicative of tricuspid incompetence. Normal flow patterns were seen during diastole *(D)*. The M-mode echocardiogram *(oblique arrow)* was used to time the diastole and systolic phases of the fetal cardiac cycle. The *horizontal arrow* denotes the position of the Doppler sample volume on the M-mode. *B*, Doppler baseline; *TV*, tricuspid valve.

ious cardiac structures were identified, and standard or modified two-dimensional echocardiographic planes were obtained. Real-time two-dimensional echo-directed M-mode imaging was performed in order to determine atrial and ventricular rates. For pulsed Doppler examination, the Doppler sample volume was placed at the appropriate intracardiac and intravascular locations with the cursor line positioned nearly parallel to the expected direction of flow. Doppler shift signals were then obtained from the target location. In order to compensate for fetal movement, the position of the Doppler sample volume was verified frequently by real-time two-dimensional echocardiographic examination. All cardiac valves were examined for regurgitation placing the Doppler sample volume in the appropriate outflow tract.

The method for estimating fetal ventricular output has been reported elsewhere from our laboratory.[7] The inner diameter of the main pulmonary artery or the ascending aorta at the target area were measured by real-time two-dimensional echocardiography. The Doppler beam was aligned with the vessel wall and

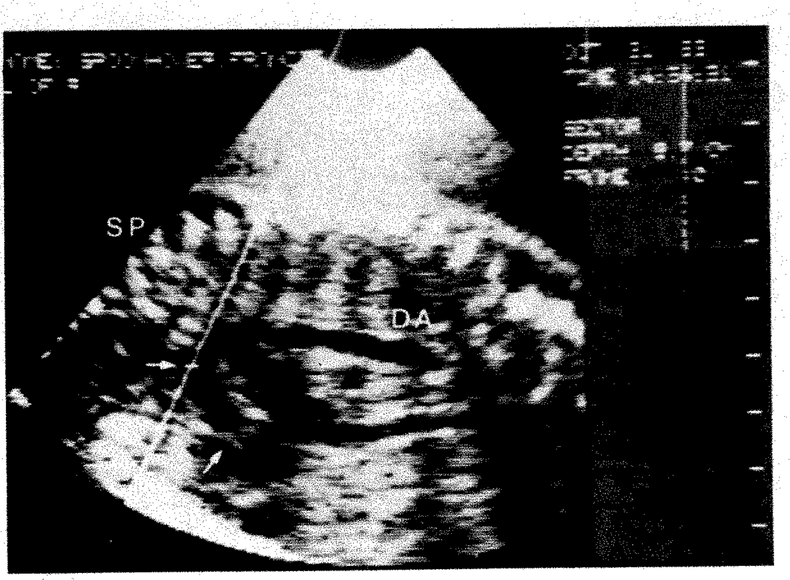

Fig. 4A. Doppler measurement of left ventricular output in a fetus with complete heart block and heart failure. The whole of the thoracic aorta was shown *(left)* in this fetus from the aortic valve level *(oblique arrow)* to the distal descending aorta. The Doppler cursor line was kept nearly parallel to the walls of the aorta at the level of the Doppler sample volume to record maximal frequency shifts from the aortic arch. *DA*, Descending aorta; *SP*, fetal spine.

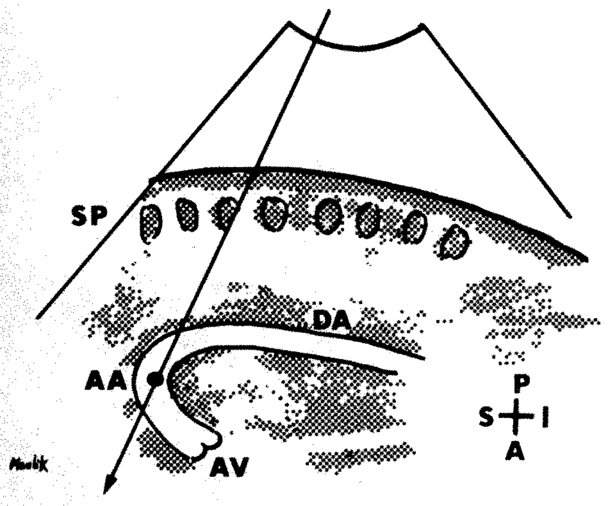

Fig. 4B. Doppler measurement of left ventricular output in a fetus with complete heart block and heart failure. Schematic diagram of the two-dimensional echocardiogram. Spatial orientation is indicated on the right (*A*, anterior; *I*, inferior; *P*, posterior; *S*, superior). *AA*, ascending aorta; *AV*, aortic valve; *DA*, descending aorta; *SP*, fetal spine.

placed in the appropriate location to obtain maximum Doppler frequency shift. The ventricular stroke volume was then estimated by integrating the instantaneous maximum velocity curve over one cardiac cycle and multiplying by the vessel cross-sectional area. This formulation assumes that the velocity profiles in the fetal aorta and the main pulmonary artery are flat and that the vascular circumference remains circular throughout the cardiac cycle.

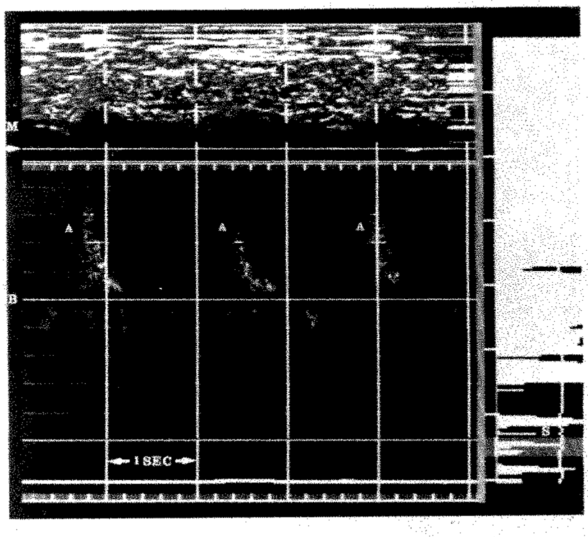

Fig. 4C. Doppler measurement of left ventricular output in a fetus with complete heart block and heart failure. Phasic flow signals *(A)* with rapid acceleration and deceleration slopes recorded on the Doppler tracing represent aortic flow *(right)*. Since the flow was directed upward (toward the transducer), it was recorded above the Doppler baseline *(B)*. Left ventricular output can be measured by calculating the area under the aortic maximum flow velocity curve if the inner aortic diameter can be measured reliably at the level of the Doppler sample volume on the two-dimensional echocardiogram.

Results

Table I summarizes the cardiac findings in nine fetuses in which prenatal echocardiography was followed by confirmatory echocardiographic evaluation in the neonatal period. Salient echocardiographic findings in

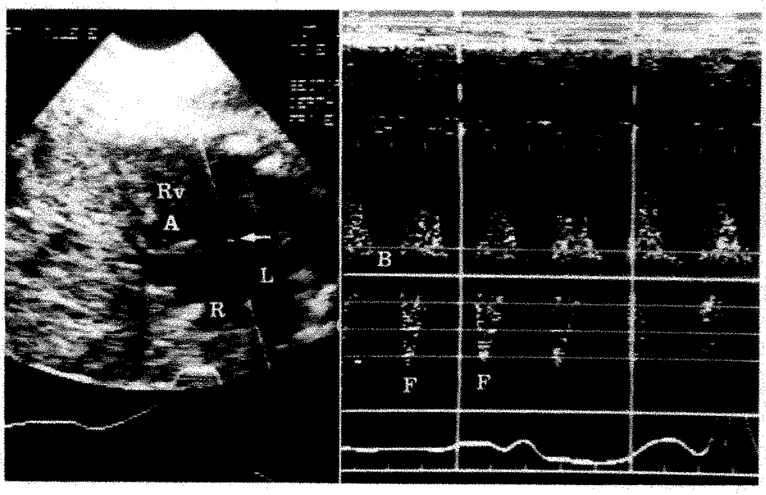

Fig. 5. Fetal Doppler echocardiographic examination in tetralogy of Fallot and aneurysmal dilatation of the main pulmonary artery. The large echo-free space present on the left of the aorta on real-time two-dimensional examination *(left)* was demonstrated by pulsed Doppler to be vascular in nature, since pulsatile flow *(right)* similar to that obtained in the pulmonary artery could be demonstrated within it *(right)*. This Doppler finding helped us make a confident diagnosis of aneurysmal dilatation of the main pulmonary artery. The *arrow* denotes the location of the Doppler sample volume. *A*, aorta; *B*, Doppler baseline; *F*, Doppler waveforms; *L*, left branch of the pulmonary artery; *R*, right branch of the pulmonary artery; *RV*, right ventricle.

one fetus with complete heart block are depicted in Figs. 1A to 4C. The ventricular rate varied between 36 to 60 bpm and the atrial rate between 120 to 150 bpm. Tricuspid regurgitation in the presence of a structurally normal tricuspid valve was also documented in the same fetus (Fig. 5). Other valves did not show any evidence of incompetence. Regurgitant flow was not noticed in any other fetus. At 38 weeks of gestation the left ventricular stroke volume measured 13.5 ml (aortic diameter, 0.7 cm; peak velocity, 175 cm/sec). As the fetal ventricular rate was 36 bpm on this occasion, the left ventricular output was estimated to be 486 ml/min. This fetus also demonstrated large echo-free spaces behind the left ventricle, indicative of pericardial effusion in the setting of congestive heart failure.

In another fetus, demonstration of a pulsatile flow in a large echo-free space in the pulmonary artery area helped us to make a confident diagnosis of aneurysmal dilatation of the pulmonary artery. Real-time two-dimensional echocardiography demonstrated a thick pulmonary valve and a small echo dropout area in the interventricular septum. However, Doppler echocardiography could not be used in this fetus to confirm the presence of ventricular septal defect because of excessive fetal movement preventing proper positioning of the Doppler sample volume in the echo dropout area. Echocardiographic evaluation of the neonate confirmed the presence of the aneurysm and demonstrated tetralogy of Fallot with a small ventricular septal defect. In three patients with ventricular ectopy, a 30% to 50% drop in the right ventricular stroke volume was noted during the ectopic beats. Since paroxysmal ventricular tachycardia was not present, minute output was not compromised in any of these fetuses. In four fetuses no cardiac abnormalities were noted during prenatal or postnatal echocardiographic evaluation.

Comment

Our study demonstrates the potential of the pulsed Doppler technique in complementing two-dimensional and M-mode echocardiography for comprehensive evaluation of fetal cardiac disorders. It was also capable of identifying tricuspid regurgitation, which was noted in one patient with complete heart block and congestive heart failure. As would be expected with this condition, the stroke volume was noted to be high in order to maintain the minute output. In another fetus with the pulmonary artery aneurysm and the postnatal diagnosis of tetralogy of Fallot, Doppler echocardiography enabled a more reliable identification of anatomic abnormalities of the fetal heart. The validity of this technique in the diagnosis of ventricular septal defect is not clearly established. Although absence of Doppler turbulence helped exclude the presence of ventricular septal defect in all patients with an echo dropout area in two-dimensional echocardiogram, this lesion may be missed in a fetus with equal ventricular pressure and virtual absence of any shunting. In the only patient with a ventricular septal defect in our series, the value of Doppler echocardiography in making the diagnosis could not be confirmed because excessive fetal movement precluded accurate placement of the Doppler sample volume in the septal echo dropout area. Although a significant reduction in the stroke volume was noted during an ectopic beat in patients with ventricular ectopy, no compromise in the minute output was observed. It may be noted that measurement of absolute flow based on flow velocity measurement by Doppler ultrasound is prone to errors contributed by inaccuracies in the measurement of vessel cross-sectional area and angle of beam incidence on the blood vessel. Therefore the application of this technique is particularly useful in observing relative changes in flow velocity, such as during an ectopic beat, when the fetus acts as its own control.

In conclusion, fetal Doppler echocardiography has potential clinical utility in more accurately assessing fetal cardiac disorders, especially as a complement to two-dimensional and M-mode echocardiographic techniques. However, further comprehensive experience is required before its precise clinical utility can be clearly defined.

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Discussion

DR. JOHN T. QUEENAN, Washington, D. C. Doppler echocardiography is an extremely effective tool in pediatric cardiology. It demonstrates and quantitates cardiac blood flow. It quantitates shunt flow and regurgitation flow and evaluates valvular stenosis. Doppler echocardiography is very valuable in quantifying shunt flow in patent ductus arteriosus, ventricular septal defect, and measuring regurgitation in congenital malformations of cardiac valves (stenosis and insufficiency).

These investigators have attempted to extend this valuable method for neonatal cardiac investigation to the fetus. In the fetus, because of the high pulmonary artery pressure and shunting, there is not a great difference between right and left heart pressures although studies by Reed et al.[1] reveal a slight right heart dominance. There is a wide range of scatter in cardiac and great vessel velocities; however, individual fetuses vary 15% from study to study (Reed et al.). Doppler studies may not be very helpful in quantifying abnormal flow in many cardiac problems. Indeed, even in newborn infants ventricular septal defect may have very subtle signs. The murmur may not be present at birth, and the shunt may not be fully developed for 2 weeks.

Alternatively, two-dimensional echocardiography has already proved to be helpful in diagnosis and evaluation of fetal cardiac problems. This modality can show chamber size, valvular integrity, dysrhythmias, ratios of heart circumference to chest circumference, segmental myocardial thickness, and pericardial effusions.

These investigators studied 19 fetuses with two-dimensional echocardiography. M-mode cardiography, and pulsed Doppler echocardiography examination between 28 and 40 weeks. But they present comments on only nine who were examined neonatally. Of these, four had normal cardiac examinations. Five were abnormal. Three had ventricular ectopy and should have been adequately diagnosed by two-dimensional echocardiography and M-mode cardiography. It is unclear how Doppler studies helped. The same can be said of the other two abnormalities, a complete heart block with congestive heart failure and a tetralogy of Fallot. The tetralogy of Fallot should have been diagnosed with two-dimensional echocardiography and M-mode cardiography. The one aspect in which the Doppler studies were helpful was in the tricuspid regurgitation diagnosed by reversal of flow over the tricuspid valve.

Doppler echocardiography has numerous technical problems that can cause serious artifacts. Fetal movement, change in angle of the Doppler beam, and inaccurate cross-sectional measurements are but a few.

I am not confident the authors in this series have proved that Doppler echocardiography has clinical utility in assessing fetal cardiac disorders. But, certainly, further investigation in a larger number of fetuses with cardiac disorders is in order to explore the potential of Doppler echocardiography.

I have two questions for Dr. Maulik: (1) Which fetal congenital cardiac disorders do you believe would benefit most from Doppler studies? (2) What effect does time in pregnancy have on ability to find abnormal flow, keeping in mind that Dr. Reed's patient with a tetralogy of Fallot had a normal examination at 24 weeks' gestation?

In conclusion, I thank Dr. Maulik for sending me his paper well in advance. I enjoyed reading it and look forward to hearing of future work.

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Obstetrical Society, third annual meeting, September 5-8, 1984.

Dr. Katherine Reed, Tucson, Arizona. I would like to emphasize a couple of points that Dr. Maulik made. I do think Doppler echocardiography is useful clinically in certain abnormalities.

I would like to use it more frequently in the detection of β-arrhythmias in order to learn more about fetal cardiac activity, but with regard to its clinical usefulness, the vast majority of these arrhythmias are benign. They disappear in the neonatal period. At this time we cannot justify using it except for study purposes.

I would also like to reiterate that I do not think at this point it is precise enough to quantitate cardiac output. I think it gives us a qualitative estimate of what is going on, and I hope with refinement of two-dimensional and Doppler techniques that eventually we will be able to say more about cardiac output. It is still not that precise in the neonate, and the fetus is even harder to get to as far as defining position and so on.

I would very much like to be able to say that this fetus with intrauterine growth retardation has decreased cardiac output or increased cardiac output. I do not think we are at that point.

Dr. Maulik (Closing. I thank Dr. Queenan for his illuminating review.

Doppler echocardiography at the present stage cannot, and it is not our desire to, replace two-dimensional echocardiography. In fact, its reliability is very much dependent upon the quality of the two-dimensional echocardiography. In the diagnosis of abnormal cardiac status, its true value lies in detecting abnormal hemodynamics and thereby complementing two-dimensional and M-mode techniques.

Experience in this area is very limited, and therefore it is difficult at present to define which particular cardiac abnormality will benefit diagnostically from this technique, except for those problems in which we expect hemodynamic alterations. It is interesting to note that we could at this moment diagnose tricuspid regurgitation or pulmonary aneurysm.

Moreover, it is fascinating in itself to investigate the cardiac hemodynamics both under normal and abnormal circumstances.

Also, in relation to the ventricular output, this fetus was followed from 33 weeks up to 38 weeks, and the output, in fact, improved after fetal digitalization via the mother.

In relation to the stage of gestation at which we can use Doppler diagnosis accurately, we have limited ourselves until recently to 28 weeks of gestation as the starting point. From that point onward we have been able to detect certain abnormalities, all of which were not included in this presentation.

Again, the question of diagnostic accuracy in early gestation cannot be answered at this moment. It is too early.

I agree with Dr. Reed's comment regarding the precise measurement of cardiac output. Even in adult and pediatric cardiology the main value lies in observing the relative changes rather than measuring the absolute flow. This is true in spite of the fact that in studies designed to verify the accuracy of the Doppler measurement of cardiac output in comparison with other existing techniques such as cardiac catherization, high correlations were observed. But it is still not a very accurate tool in terms of comparing one individual with another individual. So the main utility is more in relation to the relative changes.

Now, if we add on some of the other measurements that we can make with the Doppler technique, not absolute flow, but the characteristics of the velocity waveform itself, the technique potentially can yield an immense amount of information.

In relation to what lies in the future, I think it looks exciting. It is difficult to say how useful it would be clinically, but there are possible computer techniques for measuring moment-to-moment ventricular output and relating that to the actual changes in the vessel circumference.

There are also techniques available in which one can plot the velocity profile in a vessel cross-sectional area. And of course, there is the color Doppler instrument which can distinguish abnormalities of the flow direction; so we shall wait for the future and see how Doppler technology evolves.

Plasma volume determination in normal and preeclamptic pregnancies

Patricia M. Hays, M.D., Dwight P. Cruikshank, M.D., and Leo J. Dunn, M.D.
Richmond, Virginia

To determine the clinical usefulness of plasma volume determinations, we measured plasma volume serially throughout pregnancy with the use of the Evans blue dye-dilution technique in 20 subjects with normal pregnancies. In comparison, four preeclamptic subjects were studied. In preeclamptic subjects compared to normal subjects, plasma volume was reduced (1763 ± 216 versus 2345 ± 198 ml/m^2, $p < 0.001$). Two normotensive subjects who subsequently developed preeclampsia had reduced plasma volumes prior to the onset of hypertension as compared to volumes in those who remained normotensive (1918 ± 86 versus 2345 ± 198 ml/m^2, $p < 0.01$). Those subjects who were delivered of infants who were small for gestational age had significantly reduced plasma volumes compared to volumes of those who were delivered of infants who were appropriate for gestational age (1950 ± 333 versus 2237 ± 259 ml/m^2, $p < 0.05$), but this was true only among gravid women with pregnancies complicated by preeclampsia. We conclude that failure of plasma volume expansion is associated with the development of preeclampsia and also intrauterine fetal growth retardation in preeclamptic subjects. (Am J Obstet Gynecol 1985;151:958-66.)

Key words: Plasma volume, preeclampsia, intrauterine growth retardation

Expansion of plasma volume is a central physiologic adjustment of normal pregnancy and is closely associated with many of the other hemodynamic changes. Plasma volume begins to expand as early as 6 to 8 weeks' gestation, increases rapidly until 24 weeks, and then slowly but progressively increases between 24 and 34 weeks' gestation.[1] After 34 weeks a plateau is reached and maintained until term.[2] Chesley[3] reported that the average increase in plasma volume during a normal gestation was 42% above nonpregnant values.

Adequate plasma volume expansion is essential for optimal maternal and perinatal outcome. Conversely, failure of plasma volume to expand has been associated with various maternal and perinatal complications. Specifically, inadequate plasma volume expansion has been associated with a high risk of intrauterine growth retardation,[4] premature labor,[4] oligohydramnios,[5] chronic hypertension[6, 7] and preeclampsia.[4, 7-9] Furthermore, a marked reduction in plasma volume has been demonstrated with intrauterine growth retardation or intrauterine fetal death in pregnancies complicated by severe chronic hypertension[6, 9, 10] or preeclampsia.[7]

A controversy exists regarding the significance of inadequate maternal plasma volume expansion and its relationship to the pathogenesis of preeclampsia.[11, 12] Currently, there is general agreement that pregnancies complicated by preeclampsia and eclampsia are associated with a reduction in plasma volume, roughly in proportion to the severity of the disease. Chesley[3] reported that the average plasma volume in women with preeclampsia was 9% below expected values and was as much as 30% to 40% below normal in those with severe disease. The conflict arises over whether the reduction in maternal plasma volume is a cause or a result of the vasoconstriction. Many investigators,[3, 8, 9] however, have demonstrated that inadequate plasma volume expansion occurs before any clinical signs of preeclampsia are present.

This study was undertaken to determine the potential clinical usefulness of plasma volume determinations in predicting the development of preeclampsia or intrauterine growth retardation in apparently normal gravid women.

Material and methods

Twenty normotensive patients in good health and at various gestational ages were recruited from the prenatal clinics at the Medical College of Virginia Hospitals following detailed explanation and informed consent according to the guidelines of the Human Research Committee.

Plasma volume determinations by means of the Evans Blue dye-dilution technique[1, 13] were performed at 20 to 24 weeks' gestation (n = 9), 25 to 29 weeks' gestation (n = 11), and 30 to 34 weeks' gestation (n = 20). Of

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Table I. Maternal and perinatal outcomes

Subjects	*No.*	*Term AGA*	*Preterm AGA*	*Term SGA*	*Preterm SGA*	*Postterm LGA*
Without preeclampsia	14	9	1	3	1	—
With preeclampsia*	6	2	0	2	1	1

*Subjects with preeclampsia include those (n = 2) evaluated prospectively who subsequently developed preeclampsia and those (n = 4) who were preeclamptic at the time of plasma volume determination.

the 20 normotensive patients in whom plasma volume determinations were performed at 30 to 34 weeks' gestation, four patients were subsequently excluded from analysis for the following reasons: One was subsequently discovered to have twin gestation; in one subject a technical failure (infiltration of dye) invalidated the plasma volume measurement; in two, lipemic serum precluded accurate optical density measurement.

Plasma volume determinations were performed on an additional four patients between 33 and 42 weeks' gestation in whom the diagnosis of preeclampsia had been made. In addition, plasma volume determinations were performed on seven patients 8 weeks after an uncomplicated delivery, and these results are used as the nonpregnant values. Preeclampsia was diagnosed in those patients known to be normotensive in early pregnancy, who later demonstrated elevated blood pressure (≥140/90 mm Hg) with significant proteinuria and who became normotensive again after delivery. Intrauterine growth retardation was diagnosed when the birth weight was less than the tenth percentile for gestational age. Gestational age was assigned prospectively by dates and second-trimester ultrasound in all patients except those referred for preeclampsia who were not evaluated prospectively. In this latter group, pediatric newborn evaluation was used if dates were unknown and an ultrasound had not been obtained during the pregnancy. Preterm birth was defined as delivery at <36 weeks' gestation.

Normotensive outpatient subjects attended a special clinic between 8 and 9 AM after an overnight fast for measurement of plasma volume, blood pressure, and hematocrit as well as routine antenatal evaluation. Preeclamptic patients who were hospitalized had plasma volume determinations performed either following an overnight fast or during the intrapartum period, depending upon the severity of the disease process. All patients rested in the left lateral decubitus position for 30 minutes before and during the plasma volume measurements. A 10 ml sample of blood was obtained as a baseline sample and 2 to 3 ml of Evans blue dye (22.6 gm, United States Pharmacopeia) was injected intravenously and flushed with 10 ml of normal saline solution. Subsequently, three samples of blood were withdrawn without stasis at 10-minute intervals from an antecubital vein in the opposite arm. The samples were centrifuged immediately and the serum separated. The dye samples were compared to the patient's control serum at 620 nm with the use of a Beckman Acta CIII spectrophotometer. Each of the samples was run in triplicate and the results were then averaged.

A standard curve was generated for every lot of Evans blue dye, with concentration plotted against optical density. The optical densities of the samples were read against this standard curve to provide the actual concentration of dye in the subject's serum. The apparent plasma volumes were then calculated with the simple formula:

$$\text{Plasma volume (ml)} = \frac{\text{dye injected (mg)}}{\text{concentration of dye (mg/ml)}}$$

where plasma volume equals the milligrams of dye injected divided by the concentration of dye either obtained from the optical density value in the serum sample taken at 10 minutes after injection or determined from the optical density value extrapolated back to time zero. All plasma volume determinations reported in this study are based on the 10-minute samples except where explicitly stated.

Plasma volume can be related to the subject's height (milliliters per centimeter), weight (milliliters per kilogram), and body surface area (milliliters per square meter) according to the formula of Dubois and Dubois.[14] Rovinsky and Jaffin[2] reported that the smallest standard deviation (2.6%) and lowest coefficient of variability were obtained when plasma volume was related to body surface area instead of height or weight alone (5.5% and 6.6%, respectively). Therefore, plasma volume results in this study are expressed as a function of the subject's body surface area except where explicitly stated to be otherwise.

The results were analyzed for statistical significance by Student's *t* test. A difference between groups was considered significant if the p value was less than 0.05. Linear regression equations and correlation coefficients were calculated for fetal weight and plasma volume results.

Results

Table I summarizes the maternal and perinatal outcomes for all of the patients in whom plasma volumes were determined either at 30 to 34 weeks' gestation or

Table II. Comparison of clinical characteristics*

Group	Maternal age (yr)	Parity	Mean arterial pressure (mm Hg)		Gestational age at delivery (wk)	Birth weight (gm)
			Third trimester	At delivery		
Normotensive (n = 14)	22.4	1.1	78	84	38.2	2856
AGA (n = 10)	21.3	0.6	79	86	38.2	3092
SGA (n = 4)	25.2	2.2	74	79	38.1	2265
Preeclamptic (n = 6)	24.0	0.2	81	120	38.3	2738
AGA (n = 2)	21.0	0.0	79	110	40.8	3300
SGA (n = 3)	28.3	0.3	84	112	35.1	1603
LGA (n = 1)	17.0	0.0	76	133	42.5	5018

*Values are means.

at the time preeclampsia was diagnosed. Although 20 patients underwent plasma volume determinations at 30 to 34 weeks, four patients were excluded for reasons previously mentioned. Of the remaining 16 patients, 14 remained normotensive, and two patients (12%) subsequently developed preeclampsia.

Of the 14 patients who remained normotensive throughout the pregnancy, nine were delivered of infants who were appropriate for gestational age (AGA) at term, one was delivered of a preterm AGA infant, three were delivered of infants who were small for gestational age (SGA) at term, and one was delivered of a preterm SGA infant.

The two patients who subsequently developed preeclampsia in whom plasma volumes were determined at 30 to 34 weeks were delivered of AGA infants at term. Plasma volume determinations were performed on an additional four patients after preeclampsia had been diagnosed. Of these patients, two were delivered of term SGA infants, one was delivered of a preterm SGA infant, and one was delivered of a postterm large-for-gestational age (LGA) infant.

Table II summarizes the clinical characteristics of those patients who remained normotensive (n = 14) throughout pregnancy and those who subsequently developed preeclampsia or were preeclamptic at the time of plasma volume determination (n = 6). Both groups are further subdivided into those subjects who were delivered of AGA, SGA, or LGA infants.

Results of the plasma volume determinations for those women who remained normotensive throughout gestation and were delivered of term AGA infants are shown in Fig. 1. There was a significant increase in plasma volume from nonpregnant values (1604 ± 199 ml/m^2) to those at 20 to 24 weeks' gestation (2067 ± 184 ml/m^2, $p < 0.001$) and a further significant increase at 25 to 29 weeks' gestation (2353 ± 295 ml/m^2, $p < 0.05$). This expansion in plasma volume was maintained at 30 to 34 weeks' gestation (2345 ± 198 ml/m^2).

Fig. 1 also demonstrates the similarity between plasma volumes calculated from the 10-minute sample and that value extrapolated back to time zero. The coefficient of determination between plasma volume calculations based on a single timed sample (10 minutes) and that value extrapolated back to time zero from serial blood sampling was 97.3%. Thus our data reconfirm the reliability and validity of the single timed sample methodology.[13, 15]

In Fig. 2, the plasma volume determinations at 30 to 34 weeks' gestation in those patients who subsequently developed mild preeclampsia and were delivered of AGA infants at term (n = 2) are compared to those patients who remained normotensive during pregnancy and were delivered of AGA infants at term (n = 9). The mean plasma volume at 30 to 34 weeks was 1918 ± 86 ml/m^2 in those patients who subsequently developed preeclampsia and 2345 ± 198 ml/m^2 in those patients who remained normotensive throughout pregnancy ($p < 0.01$). The mean plasma volume findings expressed as a function of height and weight in the normotensive gravid women were 58.9 ± 4.4 ml/kg and 25.4 ± 3.3 ml/cm. These values were significantly different from plasma volume findings in those patients who subsequently developed preeclampsia (51.3 ± 0.7 ml/kg and 19.8 ± 1.3 ml/cm, $p < 0.05$).

The differences between the plasma volume determinations in milliliters per square meter in the normotensive group and in the various groups of preeclamptic patients are shown in Fig. 3. Group 1 represents those patients (n = 9) in whom plasma volumes were obtained at 30 to 34 weeks' gestation who remained normotensive and were delivered of AGA infants at term. Group 2 represents those patients (n = 2) in whom plasma volumes were obtained at 30 to 34 weeks' gestation and who subsequently developed preeclampsia. Group 3 represents those patients (n = 4) whose pregnancies were complicated by preeclampsia and in whom the plasma volume measurements were obtained at the time of diagnosis, varying between 33.5 and 42.5 weeks' gestation. Finally, group 4 represents all patients (n = 6) whose pregnancies

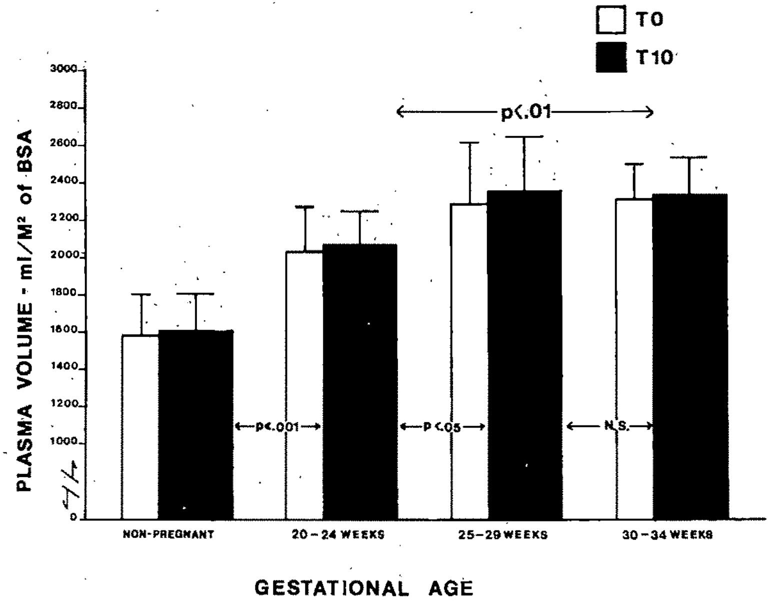

Fig. 1. Serial plasma volume (milliliters per square meter, mean ± 1 SD) obtained throughout gestation in subjects who remained normotensive and were delivered of term AGA infants. *T0* = Plasma volume calculations with the use of the concentration of dye determined from the optical density value extrapolated back to time zero. *T10* = Plasma volume calculations with the use of the concentration of dye obtained from the optical density value in the serum sample taken at 10 minutes after injection.

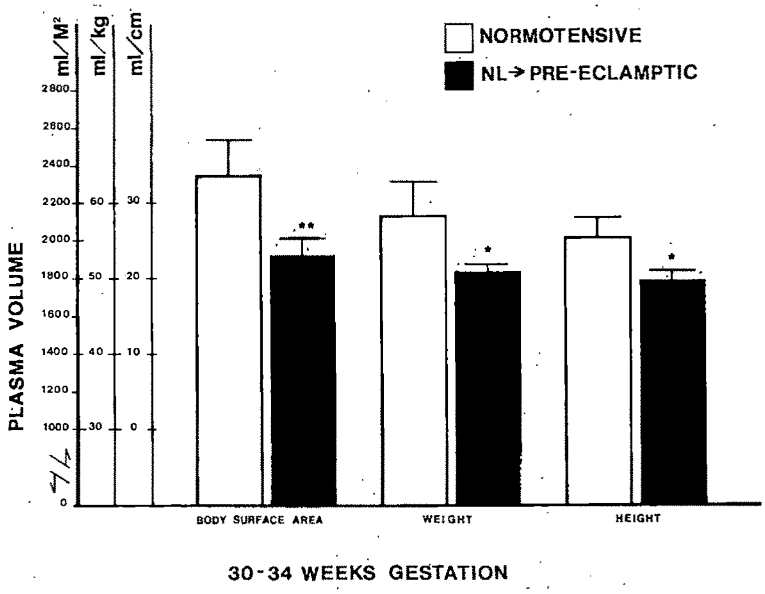

Fig. 2. Comparison of plasma volume determinations (milliliters per square meter, milliliters per kilogram, milliliters per centimeter) at 30 to 34 weeks' gestation in those patients who remained normotensive and were delivered of AGA term infants (*open bars*, n = 9) and those who subsequently developed preeclampsia and were delivered of AGA term infants (*solid bars*, n = 2). $^*p < 0.05$. $^{**}p < 0.01$.

were complicated by preeclampsia including those evaluated prospectively who subsequently developed preeclampsia and those who were preeclamptic at the time of plasma volume determination.

The significant difference in the plasma volumes at 30 to 34 weeks' gestation between groups 1 and 2 ($p < 0.01$) has been previously discussed. The mean plasma volume in pregnancies already complicated by preeclampsia (group 3) was 1660 ± 224 ml/m², significantly less than the plasma volume findings in normotensive patients at 30 to 34 weeks (2345 ± 198 ml/m², $p < 0.001$). Not surprisingly, when groups 2 and 3

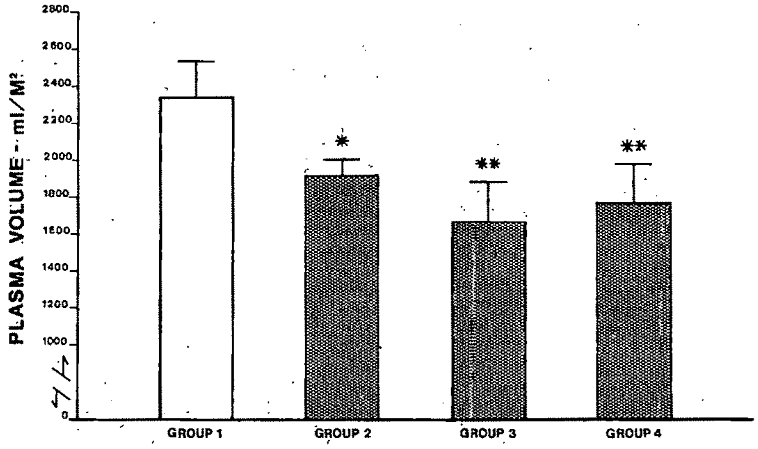

Fig. 3. Relationship between plasma volume findings and preeclampsia. Group 1 represents nine normotensive subjects at 30 to 34 weeks. Group 2 represents two normotensive subjects at 30 to 34 weeks' gestation who subsequently developed preeclampsia. Group 3 represents four subjects who were preeclamptic at the time of plasma volume determination. Group 4 represents the mean plasma volume in all six patients in groups 2 and 3. $^*p < 0.01$. $^{**}p < 0.001$.

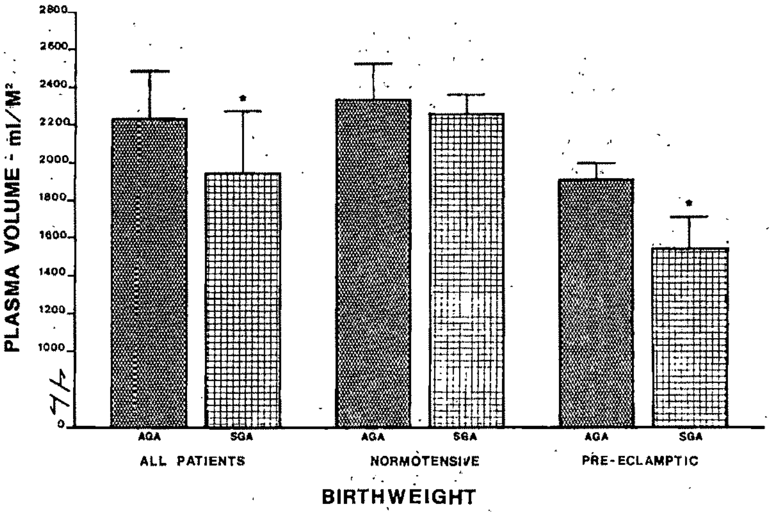

Fig. 4. Plasma volume findings in all patients who were delivered of AGA infants *(diagonal crosshatch)* are compared to all patients who were delivered of SGA infants *(horizontal crosshatch)* regardless of duration of gestation and presence or absence of preeclampsia. Similar comparisons for both the normotensive and preeclamptic subjects who were delivered at term are depicted. $^*p < 0.05$.

are combined, there is a marked difference in the plasma volume findings between those patients who either eventually developed or had preeclampsia (group 4, 1763 ± 216 ml/m^2) and those patients who remained normotensive ($p < 0.001$).

The plasma volume findings in patients who were delivered of AGA infants are compared with the findings in those patients who were delivered of SGA infants in Fig. 4. The mean plasma volume was 2237 ± 259 ml/m^2 in all patients who were delivered of AGA infants ($n = 12$) and 1950 ± 333 ml/m^2 in all patients who were delivered of SGA infants ($n = 7$) regardless of duration of gestation and the presence or absence of preeclampsia ($p < 0.05$). Fig. 4 also compares the relationship between the plasma volume findings, intrauterine growth retardation, and the presence or absence of preeclampsia. Although a significant relationship ($r = 0.53$, $p < 0.05$) was found between plasma volume at 30 to 34 weeks' gestation and the birth weight of infants among the normotensive patients, no significant difference was demonstrated between the plasma volume findings at 30 to 34 weeks' gestation in the normotensive patients who were delivered of AGA infants at term (2345 ± 198 ml/m^2, $n = 9$) and normotensive subjects who were delivered of SGA infants at term (2262 ± 97 ml/m^2, $n = 3$). However, the mean plasma volume of 1918 ± 86 ml/m^2 in those subjects who developed preeclampsia and were delivered of AGA infants at term ($n = 2$) was significantly higher than the 1550 ± 163 ml/m^2 in those patients who had preeclampsia and were delivered of SGA infants at term ($n = 2$) ($p < 0.05$).

Comment

Reduction of plasma volume in pregnancies complicated by preeclampsia is now a well-established association. Several investigators have confirmed the association between preeclampsia and relative maternal hypovolemia as reflected in plasma volume determination.[3, 4, 7-9]

More recently, however, others have demonstrated that failure of plasma volume expansion or actual contraction of plasma volume precedes the appearance of preeclampsia. Blekta et al.[8] demonstrated significantly reduced blood and plasma volumes in nine patients at 26 weeks' gestation, prior to the development of preeclampsia. Chesley,[3] quoting Duffus and MacGillivray, stated that six of seven apparently normal patients who subsequently developed preeclampsia had relatively low plasma volumes at 30 weeks, preceding the appearance of any clinical signs or symptoms. Among gravid women with chronic hypertension, however, Sibai et al.[15] could not demonstrate a correlation between plasma volume determinations performed during the third trimester and the subsequent development of superimposed preeclampsia. In 1979, Gallery et al.[9] reported on the plasma volumes findings in 229 normotensive women, of whom 40 subsequently became preeclamptic. They documented an actual reduction in plasma volume in 29 of the 40 women prior to the onset of hypertension. Our results confirm the earlier findings that significantly reduced maternal plasma volume precedes the clinical appearance of preeclampsia in previously normotensive patients. Of the 20 normotensive patients in our study evaluated prospectively, two subsequently developed preeclampsia. The plasma volumes determined at 30 to 34 weeks' gestation in these patients who subsequently developed preeclampsia were significantly lower than the plasma volumes of those patients who remained normotensive.

Previous investigators have demonstrated a direct relationship between the degree of plasma volume expansion and the birth weights of infants.[1] Among gravid women with chronic hypertension, fetal growth appears to be proportional to plasma volume expansion. Arias[6] demonstrated that the failure to achieve a blood volume of at least 60 ml/kg clearly identified those pregnancies complicated by chronic hypertension to be at increased risk for intrauterine growth retardation and fetal death. Sibai et al.[10, 15] found a significant difference in the plasma volume among gravid women with chronic hypertension who were subsequently delivered of AGA infants and those who were delivered of SGA or stillborn infants. Gallery et al.[9, 16] reported a significant relationship between the plasma volume determined at 33 to 36 weeks and the eventual birth weight of the infants among normotensive gravid women and those with chronic hypertension. Gallery et al.[9] also demonstrated that the birth weights of infants born to those women who subsequently developed preeclampsia were slightly lower ($p < 0.05$) than those of infants born to women who remained normotensive, although this difference may have been attributable to differences in gestational age at delivery. Goodlin et al.[4] found significantly lower maternal plasma volumes in pregnancies complicated by intrauterine growth retardation. Of the nine women with growth-retarded fetuses, six (68%) had plasma volume values greater than 3SD below the mean (48 ml/kg). Of note, the plasma volumes were not determined prospectively but rather after the obstetric complication had been identified.

Our results indicate that although there is a significant difference in maternal plasma volume between gravid women who were delivered of AGA infants and those who were delivered of SGA infants, this difference is attributable to the association between intrauterine growth retardation and preeclampsia. The apparent disagreement with the findings of Goodlin et al.[4] of low maternal plasma volumes in normotensive pregnancies complicated by intrauterine growth retardation may be a function of the time at which the plasma volume measurements were performed, the limited number in our study population, or the severity of the intrauterine growth retardation.

In conclusion, normal plasma volume expansion during pregnancy is associated with optimal maternal and perinatal outcome. Conversely, failure of plasma volume expansion is associated with preeclampsia and more importantly precedes the clinical appearance of the disease. Plasma volume contraction is associated with a risk of intrauterine growth retardation but in our study only among gravid women with pregnancies complicated by preeclampsia.

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Discussion

DR. ROBERT C. GOODLIN, Omaha, Nebraska. In my review of the titles presented to this Society during the 1940s and 1950s, pregnancy-related plasma volume expansion was the single most frequent obstetric subject. While these past investigators recognized the limitations of their techniques by speaking of the "Evans blue space" and not plasma volume values per se, none of these studies attempted to relate pregnancy outcome to the failure of excess of plasma volume. The authors in the present study have serially determined the plasma volumes in a relatively small number of pregnant women. Some of their interpretations I understand, and others I do not. Those which I do not understand include their technique of measurement of plasma volume with Evans blue. As pointed out by Longo and Hardesty,[1] the Evans blue technique is imprecise for estimating plasma volume. Campbell and MacGillivray,[2] as well as ourselves, have shown that women with preeclampsia have a faster rate of disappearance of Evans blue from the serum. The laboratory can often recognize the preeclamptic patient simply by the abnormal rate of the disappearance of Evans blue. For this reason, I cannot accept a single timed determination for Evans blue concentration, even if the technique is satisfactory in the normal subject. I am also unable to understand how we can use surface area as a basis for comparison of plasma volumes in pregnant women. Most investigators, including the present authors, have used a formula for calculating surface area from height and weight derived by DuBois and DuBois.[3] These latter investigators clearly stated that their formula had much greater error for predicting surface area in people of unusual body shape. We[4] continue to compare plasma volume on a weight basis and correct for the very thin or the markedly obese patient.

For those of us who believe in the importance of plasma volume expansion, it is encouraging that the authors have again found that the relative maternal hypovolemia precedes the onset of symptoms of preeclampsia. However, it was disappointing to learn that they were unable to correlate degree of plasma volume expansion with fetal weight.

The one relatively constant relationship in obstetrics, whether it be in humans, farm animals, or birds, is that the weight of the neonate is most closely related with the degree of maternal plasma volume expansion.[4] The growth-retarded fetus is very likely to be associated with maternal relative hypovolemia and, in humans, with the appearance of preeclampsia. The terms small for gestational age and intrauterine growth retardation are, in my opinion, confusing. I believe we would understand the problem better if we could return to the concept of dysmaturity as defined by Sjostedt et al.[5] in 1958. Dysmaturity is associated with oligohydramnios and maternal hypovolemia and subsequent developmental problems. The term avoids inclusion of the genetically "small people syndrome" or infants born of small parents, as well as many other normal small newborn infants. In the authors' study, the two SGA newborn infants of mothers without hypovolemia were above the fifth percentile in weight. I wonder whether there was associated oligohydramnios and what were the sizes of their parents?

The important issue is what, if any, is the significance of failure of maternal plasma volume expansion. The Europeans have demonstrated that the spiral arteries are dilated between the eighteenth and nineteenth weeks of pregnancy by invasion of trophoblastic cells; when this invasion does not occur, there is subsequent fetal growth retardation and sometimes preeclampsia.[6] Our studies indicate that women with failure of expansion of plasma volume have growth-retarded fetuses and/or preeclampsia or premature labor. Based on the velocity profiles of the arcuate arteries as described by Campbell et al.,[7] they presumably have failure of dilatation of the spiral arteries. While the basic abnormality may be an anatomic one of the failure of the spiral arteries to dilate, it is still appropriate to treat the associated failure of plasma volume expansion in order to prevent the related maternal complications such as preeclampsia and/or premature labor (Fig. 1).

I congratulate the authors on their study and their good luck. We have prospectively studied more than 100 apparently normal pregnant women and have failed to find as many abnormal pregnancies as the authors apparently found in their prospective study of only 20 pregnant women. I am curious as to how they achieved this high degree of subsequent abnormality in apparently normal pregnant women.

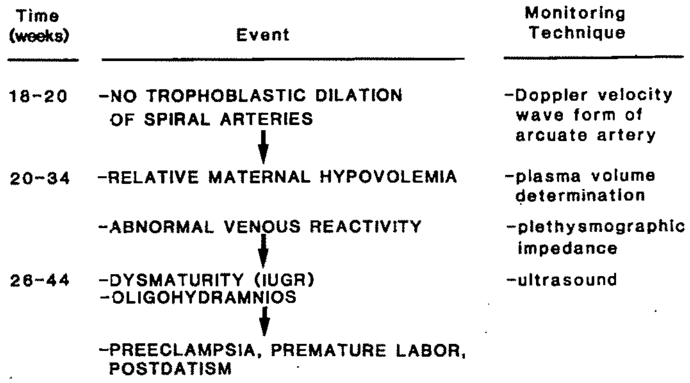
Time (weeks)
Event
Monitoring Technique
18-20
-NO TROPHOBLASTIC DILATION OF SPIRAL ARTERIES
-Doppler velocity wave form of arcuate artery
20-34
-RELATIVE MATERNAL HYPOVOLEMIA
-plasma volume determination
-ABNORMAL VENOUS REACTIVITY
-plethysmographic impedance
26-44
-DYSMATURITY (IUGR)
-OLIGOHYDRAMNIOS
-ultrasound
-PREECLAMPSIA, PREMATURE LABOR, POSTDATISM

Fig. 1. Pattern of abnormal gestational events.

The authors have been careful to refrain from discussing the therapeutic implications of their study. I should appreciate learning of their recommendations (if any) to an apparently normal pregnant woman who at 26 weeks is found to be hypovolemic.

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DR. JAMES SCOTT, Salt Lake City, Utah. Dr. Hays, I noticed in the first sentence of your abstract that your purpose was to determine the potential clinical usefulness of the test.

Along those lines, I wonder if you could discuss the clinical importance versus the statistical significance of the study. In other words, how many false positive or false negative results did you have (in individual patients) in the normal group versus the preeclamptic group? Did you have patients who had normal plasma volumes who were preeclamptic and vice versa?

Second, if you do have a predictive test (or anyone has a predictive test) for the development of preeclampsia, what do you do with that information? Have you tried anything to alter the plasma volume in these patients?

DR. PRESTON DILTS, Memphis, Tennessee. First of all, you said there is a controversy about whether plasma constriction is cause or effect in relation to vasospasm. It seems to me there is perhaps a third choice. I agree fully it is not the cause. I am not sure that it is the effect and I wonder if it is not the third choice, that both are the effect of whatever the cause of the disease is, and that is our real problem.

Second, in a somewhat larger series, we found, I think, the same thing you did. You did not define preeclampsia well enough so I cannot be sure. In our series we found that patients who developed only mild preeclampsia did not have restricted plasma volume. Those patients who became severely preeclamptic or eclamptic had constricted plasma volume as early as 26 weeks, and to answer Goodlin's and Scott's questions, we have tried a number of things and have not been able to change the outcome so far. What did your patients have, mild or severe preeclampsia?

Finally, the only other place we found low plasma volume to be predictive, perhaps similar to what you found, was in patients who have chronic hypertension. In those patients, if they have low plasma volume, in spite of most everything we do at this point in time, they will have either intrauterine growth retardation or a fetal death. Again we do not have good ways of treating other than very early delivery.

Was the lack of predictability in your patients perhaps due to the small number of patients studied, since I think you were probably dealing with only a couple of patients?

DR. E. J. QUILLIGAN, Madison, Wisconsin. Did you find any differences in your 20- to 24-week patients? Unless I missed it, you mentioned the major differences were at 30 to 34 weeks. I wondered if there were differences in plasma volumes earlier on.

DR. HAYS (Closing). First I will address Dr. Goodlin's questions.

Obviously, the controversy over whether to use single timed or multiple sampling is the reason why we did both. We did not see a difference in the behavior of the timed samples in the preeclamptic women. However, all patients underwent multiple blood sampling at 10, 20, and 30 minutes for that very reason.

How to express the data remains a question in the plasma volume literature. That is why I chose to present our data as a function of body surface area, height, and weight in an effort to try to avoid that problem as well as to show that the significant differences were the same regardless of how the data were expressed. When expressed in terms of body surface area, the differences were actually more significant ($p < 0.01$) as compared to that with height and weight ($p < 0.05$). It is interesting that Gallery uses height and that in the formula of DuBois and DuBois the exponential power is actually weighted for height. In addition, some investigators wonder whether plasma volume actually does expand proportionately as weight increases. I continue to use body surface area primarily because of the work of Rowinsky who showed that, in fact, the smallest standard deviation and smallest coefficient of variation were obtained when body surface area was used.

In response to the third question, we do have a high incidence of preeclampsia. We are in the "preeclamptic belt," so to speak. A great many of our patients are black, and they have a high incidence of preeclampsia. These patients were recruited by me, and I think perhaps that might have been a factor, possibly a biasing factor. However, the incidence of 12% for preeclampsia is in agreement with the incidence in our general population.

With regard to the hematocrits in our group, there was a definite trend, but it was not significant. In group 1, which was the normotensive group, the mean hematocrit was 33% at 30 to 34 weeks. In group 2, those patients who subsequently developed preeclampsia, it was 35%, and in those patients who had preeclampsia, it was 37%.

I could not demonstrate a significant difference in plasma volume values for normotensive patients who were delivered of AGA infants versus those with SGA infants. There was, however, a significant correlation between plasma volume and infant birth weight with an R value of 0.53 ($p < 0.05$).

I think everyone here is asking the general question what is the value of having a predictive test, and that is what I plan to look at now. To expand plasma volume with intravenous volume expanders would be the most aggressive approach and I would not use these until I could prove nothing else worked.

To answer Dr. Scott's questions, there were no patients who had normal plasma volumes who developed preeclampsia. He also asked, "How could you alter the plasma volume?" I would start with bed rest and improved nutrition. Interestingly, Gallery has presented data on patients who have chronic hypertension. She actually showed that certain β-blockers, particularly Oxyprenolol, expanded plasma volume. This raises the question of whether to use antihypertensive therapy in preeclamptic women to see if plasma volume can expand. That has not been done.

I agree with Dr. Dilts that it may not be a question of cause-and-effect but may be an associative factor. However, if something can be used to identify those patients at risk for preeclampsia, intrauterine fetal death, and intrauterine growth retardation, at least there would be a flag. I cannot help but think that one would increase fetal surveillance and perform serial ultrasound evaluations, which could very well make a difference in the obstetric management and outcome.

With regard to mild preeclampsia versus severe preeclampsia, I did not classify these. Those patients who were followed up prospectively, had decreased plasma volumes at 30 to 34 weeks' gestation, and subsequently developed preeclampsia were considered to have mild preeclampsia. There was a decrease in plasma volume at 30 to 34 weeks preceding the clinical appearance of hypertension. Those patients who had plasma volumes measured at the time of diagnosis and who also had the most contracted plasma volumes were considered to have severe preeclampsia. Of course, we all have to admit to the use of small numbers. Hopefully, the number of patients will increase.

Septicemia during pregnancy: A study in different species of experimental animals

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Münster, Federal Republic of Germany

When *Escherichia coli* B6 lipopolysaccharide, 0.2 mg/kg of body weight, was infused into nonpregnant minipigs during a 5-hour period, the animals died after 12 to 16 hours as a result of endotoxic shock. When the same infusion was given to six pregnant minipigs at term, these animals died after only 3½ hours. The decrease in the number of white blood cells, the number of platelets, hematocrit, and clotting factors was not significantly different between the two groups. The acid-base status, however, indicated a much more pronounced metabolic acidosis in the pregnant animals than in the nonpregnant controls. In the pregnant minipigs heart rate, cardiac output, mean arterial pressure, and total peripheral resistance indicated cardiovascular collapse, and the multiple wire, platinum surface electrode revealed a drastic reduction in uterine tissue oxygenation in the pregnant animals. The data support the hypothesis that pregnant animals at term are more susceptible to the harmful effects of lipopolysaccharide. Early death in the pregnant minipigs, however, was not associated with disseminated intravascular coagulation as it is in smaller animals (rat, rabbit, and hamster). (AM J OBSTET GYNECOL 1985;151:967-75.)

Key words: Endotoxemia, experimental endotoxin shock, minipigs—pregnancy as a preparatory mechanism

Apitz[1] observed during his studies on endotoxins, in 1935, that one rabbit died after application of less endotoxin than the rest of the group received, and he noted that this animal was pregnant. This observation stimulated research to substantiate the hypothetical concept that pregnancy renders the organism more susceptible to infection or products of infection.

Data accumulated in regard to the generalized Shwartzman reaction in pregnant and nonpregnant rabbits and the older literature were reviewed by McKay[2] and Selye[3]: Pregnant animals at term generally needed less endotoxin for the development of shock and organ necrosis resulting from disseminated intravascular coagulation than the nonpregnant controls.

We have previously studied smaller animals in an endotoxin infusion model,[4] and in the present study we have compared those data with results obtained under similar conditions in a larger animal, the minipig. There are very detailed studies only on the pregnant baboon[5] and sheep[6] as examples of large animals reported in the literature. We chose the minipig because we found it to be a suitable model for studies on septicemia (unpublished results). In the present study the reactions to the same dose of lipopolysaccharide are compared in pregnant and nonpregnant minipigs. The results are then contrasted to observations in smaller animals.

Material and methods

Animals. A total of 12 nonpregnant and six pregnant minipigs (Göttinger Miniaturschweine) were used. These animals are characterized by a genetically determined form of dwarfism. The weights of the animals ranged from 35 to 78 kg and their ages were between 2 and 3 years. All pregnant animals were in the last third of their 110-day gestation.

Medication. One hour before the start of the infusion the animals were premedicated by an intramuscular injection with 0.5 mg of atropine (Drobena, Düsseldorf, Federal Republic of Germany) and 120 to 200 mg of Azaperon (Jansen, Düsseldorf, Federal Republic of Germany). For the induction of anesthesia Metomidat hydrochloride (Jansen) was slowly given at a dosage of 2.5 to 10 mg/kg of body weight through a cannula placed in a vein of the pig's ear. The anesthesia was maintained by continuous administration of Metomidat hydrochloride, 5.0 to 7.5 mg/kg of body weight in 0.9% saline solution.

Preparation. A tracheotomy was performed, and after intubation the animals were ventilated on a pressure- and volume-controlled respirator.

A catheter was guided to the superior vena cava via the regular vein and was used for the administration of fluids, medications, and anesthetics, as well as for blood sampling.

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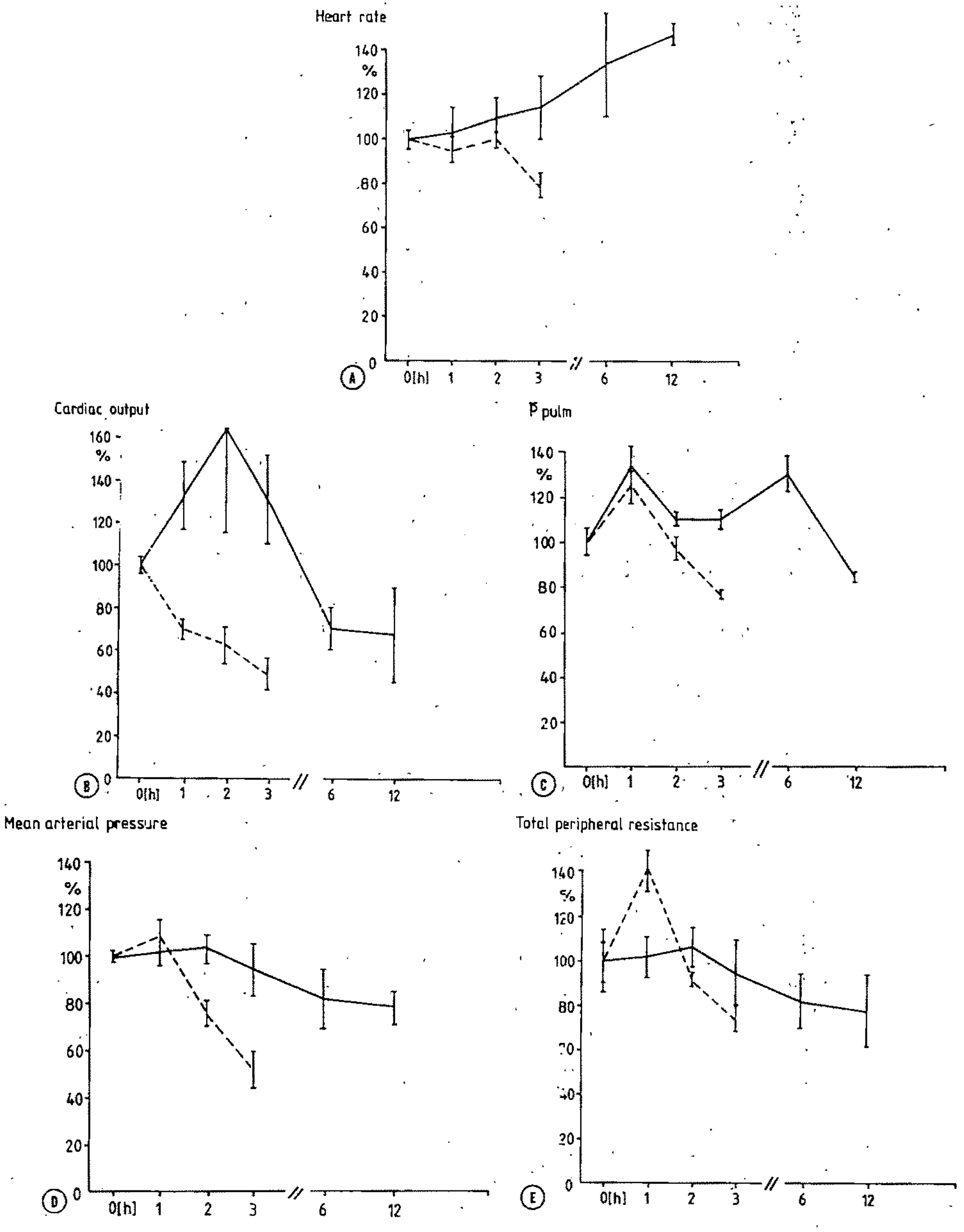

Fig. 1. Cardiovascular changes after endotoxin administration. Relative changes from baseline values are given in percentages. The *broken lines* represent pregnant animals; the *solid lines* represent nonpregnant animals.

A Swan-Ganz catheter (American Edwards Laboratories, Santa Ana, California) was placed into the pulmonary artery via the jugular vein for measurement of pulmonary artery pressure and another one via the femoral artery for measurement of the arterial pressure into the aortic arch and for sampling of arterial blood. The cardiac output was determined by the cold dilution method with a thermistor sensor placed at the catheter tip and with the use of an external measuring device (Fischer, Erlangen, Federal Republic of Germany). Urinary output was measured continuously with a Foley catheter placed before the start of the experiments.

Local oxygen pressure distribution. A multiple wire platinum surface electrode, after Kessler and Lübbers, was placed by laparotomy in pregnant animals on the uterine surface for the measurement of the tissue Po_2 distribution.[7] The glass electrode consisted of eight platinum wires with a diameter of 0.15 μm and which were isolated from each other. The silver-silver chloride reference electrode was positioned in a circular fashion around the glass core. The electrolyte space, which contained 0.2 mol/L of bromide-free potassium chloride, was covered by a Teflon membrane 12 μm thick. For mechanical stability, Cuprophan (Enka, Münster, Federal Republic of Germany) platelets were added into the electrolyte space. The low weight of the electrode (2.1 gm) prevented tissue ischemia. The electrodes were placed on the uterus with a Teflon hat, and a rubber gum ring around this device prevented diffusion of oxygen in the air to the electrode. Before and after each measurement the electrodes were calibrated in 0.9% saline solution with nitrous oxide and a mixture of 5% oxygen and 95% pure nitrous oxide (Messer-Griesheim, Federal Republic of Germany) to correct for the electrode drift. By manual movement of the electrode, about 100 to 160 individual measurements could be taken for one Po_2 histogram without damaging the tissue. In case of impaired microcirculation the computer-generated Po_2 histogram would show a loss of the normal bell-shaped distribution and a marked shift to the left, indicating tissue ischemia.[7]

Infusion experiment. After the initial blood samplings at time 0 an infusion of *Escherichia coli* B6 lipopolysaccharide (Difco Laboratories, Inc., Detroit, Michigan), 0.2 mg/kg of body weight, in 50 ml/kg of Ringer's solution, was administered by an infusion pump during 12 hours or until the death of the animals. During the experiments, heart rate, arterial blood pressures, and arterial Po_2 were measured continuously, as were urinary excretion, cardiac output, and Po_2 (by histogram) in the uterine tissue at hourly intervals.

Acid-base balance. For an assessment of the acid-base-state of the animals, the pH value was determined in the descending aorta and superior vena cava with a calibrated glass electrode. The Po_2 was measured with a Clark electrode, and the Pco_2 was determined by a Severinghaus electrode. The base excess was calculated according to the Siggaard-Andersen nomogram.

The lactate concentration in the blood was determined photometrically by measurement of the increase in reduced nicotinamide adenine dinucleotide in the enzymatic lactate dehydrogenase test (Boehringer Mannheim, Federal Republic of Germany).

Blood. The hematocrit was calculated from the electronically measured mean corpuscular volume, and the erythrocyte number was measured by a Coulter Counter. Leukocyte numbers were determined by Coulter Counter analysis, and platelet number by the thrombocounter. Plasma fibrinogen was measured in a coagulometer after application of 0 to 2 ml of thrombin solution (Behring, Marburg, Federal Republic of Germany) to citrated plasma. Clotting factors II, V, VII, X, and XII were measured with plasma deficient for the specific factor (Behring), with calcium chloride solution and prothrombin added for the determination of factor XII and the other clotting factors, respectively. Antithrombin III was measured with the use of the chromogenic substrate S 2238 (Kabi, Stockholm, Sweden).

For statistical evaluation of all results we calculated the means and standard errors of the means at 0, 1, 2, 3, 6, and 12 hours.

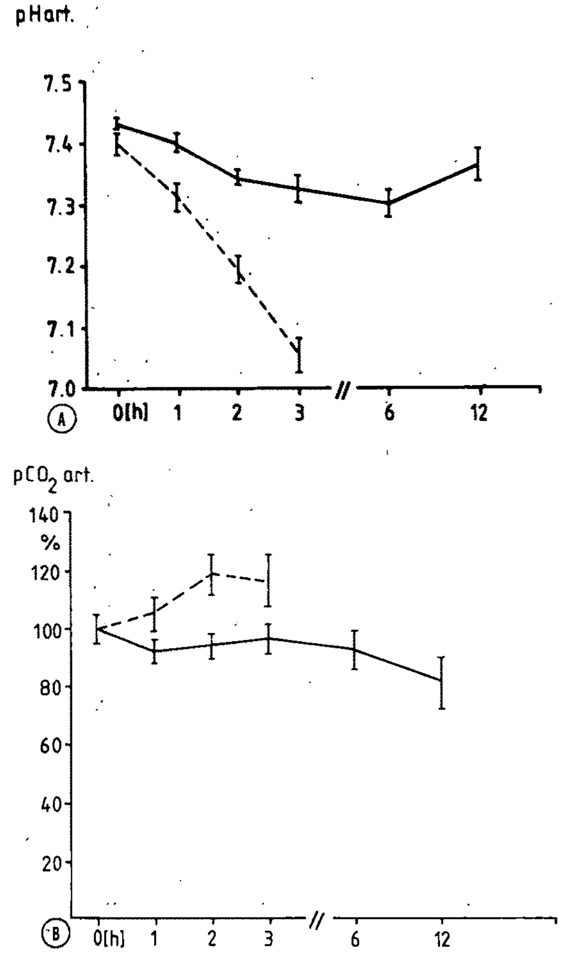

Fig. 2, *A* and *B*. Metabolic changes and acid-base status. Relative changes from baseline values are given in percentages. The *broken lines* represent pregnant animals; the *solid lines* represent nonpregnant animals.

Results

Effects of the endotoxin infusion on the animals. The average survival of the pregnant minipig was 3½ hours. None of the 6 animals lived longer than 5½ hours. In contrast the mean survival for nonpregnant minipigs was 16 hours, and four animals in this group survived the entire experiment. The earliest time of death in this group was 6 hours.

Cardiovascular effects. Within 3 hours after endotoxin infusion there was a 20% decrease in the heart rate of the pregnant animal. In the nonpregnant animal the heart rate increased by 14% during the same period (Fig. 1, *A*). Cardiac output decreased by 50% in the pregnant animal, but there was a 40% increase in the nonpregnant control (Fig. 1, *B*). Pulmonary pressure of the pregnant animal increased initially and then decreased by 20% after 3 hours. In the nonpregnant con-

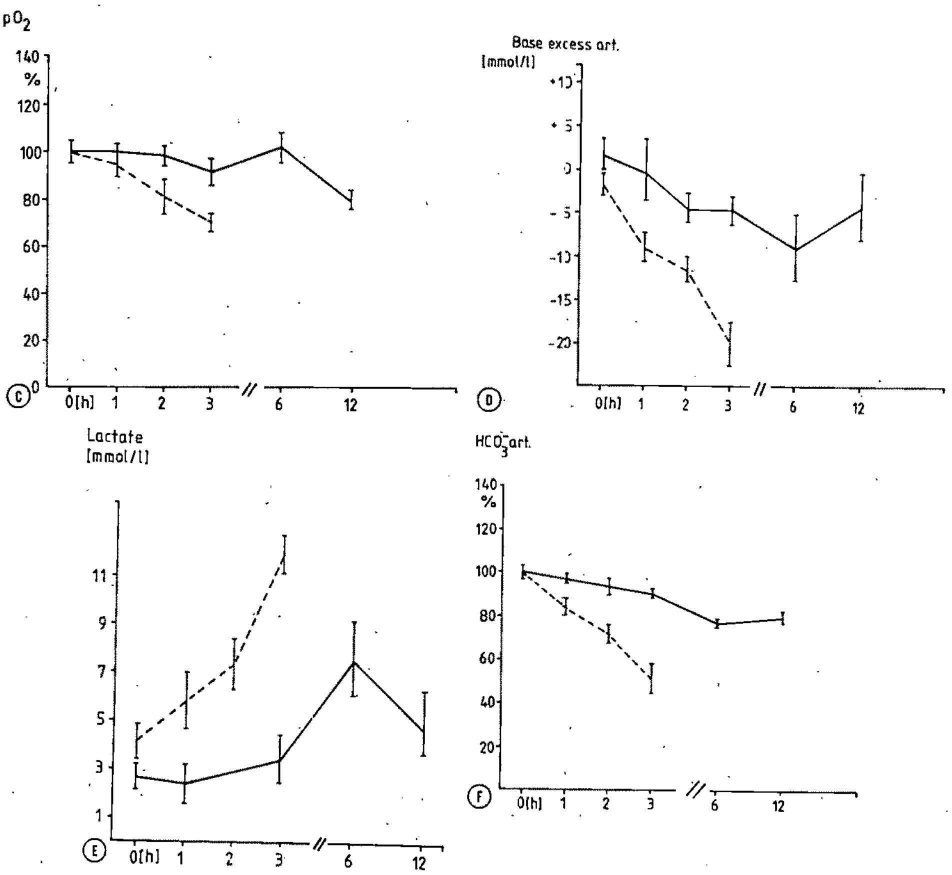

Fig. 2, *C* to *F*. Metabolic changes and acid-base status. Relative changes from baseline values are given in percentages. The *broken lines* represent pregnant animals; the *solid lines* represent nonpregnant animals.

trol the initial rise was followed by a plateau for several hours, followed by a final drop (Fig. 1, *C*). Mean arterial pressure dropped by 50% in the pregnant group; there was a delayed and less pronounced drop in the controls (Fig. 1, *D*). Total peripheral resistance in the pregnant animal revealed a 30% increase during the first hour and a subsequent drop by 25%, whereas in the nonpregnant animal it stayed fairly constant during the 12-hour experiment (Fig. 1, *E*).

Metabolic parameters. In the pregnant group of animals the arterial pH dropped from 7.40 to 7.05 within 3 hours. In the nonpregnant control group the pH remained in the normal range for 12 hours (Fig. 2, *A*). The arterial Pco_2 increased by 15% in the pregnant animals and did not change in the controls (Fig. 2, *B*). The Po_2 values decreased by 30% in the pregnant animals and remained fairly constant in the nonpregnant group of animals (Fig. 2, *C*). Base excess fell from −1.17 to −22.57 in the pregnant group of animals. In the same period of time the value decreased from 4.2 to 4.1 in the nonpregnant controls (Fig. 2, *D*). Lactate revealed a massive increase from 4.2 to 11.8 in the pregnant group but there was no significant change in the control group during the same period of time (Fig. 2, *E*). Bicarbonate dropped by 50% in the pregnant group of animals compared to 10% in the controls (Fig. 2, *F*).

Blood parameters. The drop in leukocyte number was similar in both groups (Fig. 3, *A*) and so was that of the platelets (Fig. 3, *B*) and the plasma fibrinogen concentration (Fig. 3, *C*). Hematocrit changes were not significantly different in both groups during 3 hours (Fig. 3, *D*). The decrease in the concentration of various clotting factors was similar in both groups up to 3 hours. The pregnant animals had already died before the drastic drop occurred in the nonpregnant control group.

Urinary excretion had ceased after 3 hours in all pregnant animals, whereas in the nonpregnant controls the urinary output increased for the first 3 hours and subsequently showed a gradual decrease.

Histograms. The normal Po_2 distribution measured by the multiple wire, platinum surface electrode was characterized by a symmetrical form of the oxygen partial Po_2 curve with a lack of anoxic measurement classes. This type of pattern was reproducibly seen in the non-

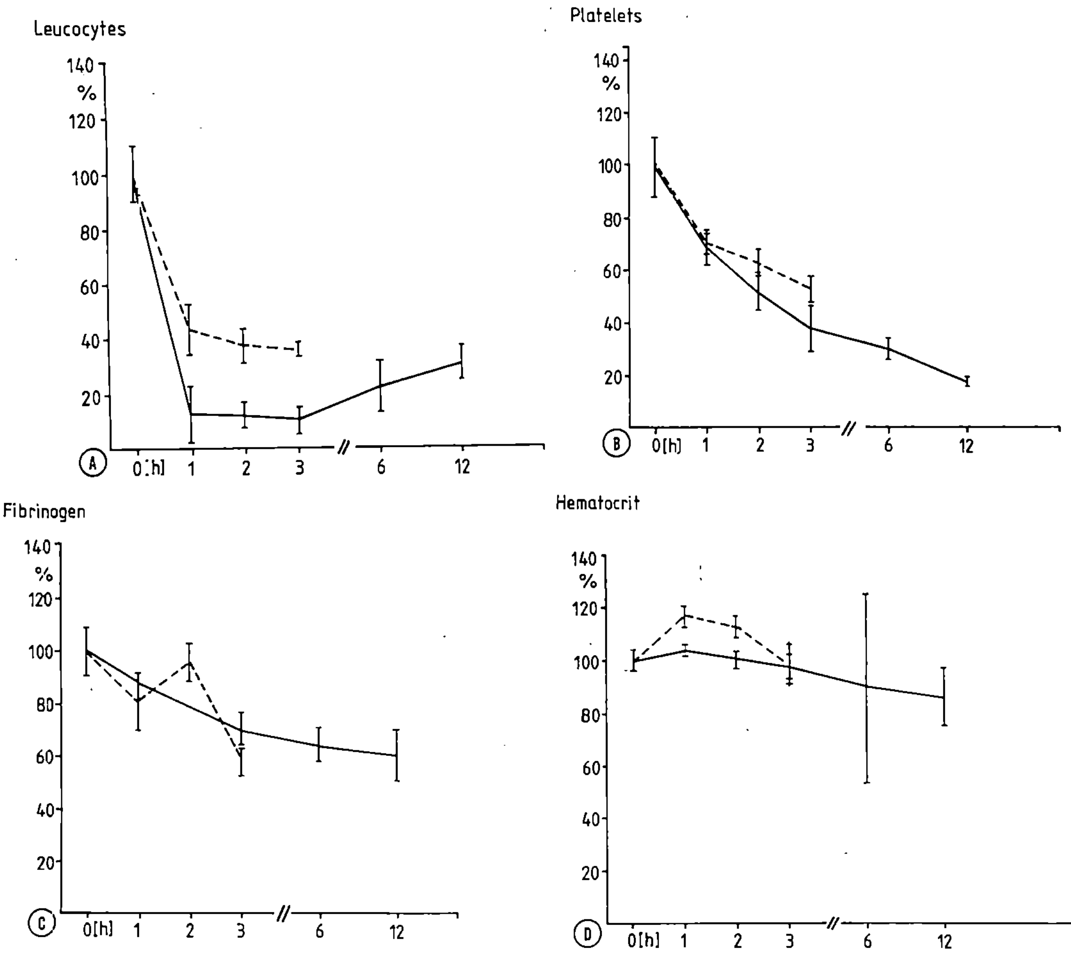

Fig. 3. Changes in blood parameters. Relative changes from baseline values are given in percentages. The *broken lines* represent pregnant animals; the *solid lines* represent nonpregnant animals.

pregnant group of animals at the beginning of the experiments and 1 to 3 hours after infusion of endotoxin. Contrary to this, in the pregnant group of animals, the Gaussian distribution of Po_2 measurements was lost 2 hours after the start of endotoxin infusion with a marked shift to the left, which indicated an increased frequency of anoxic measurement classes in the uterine tissue.

Comment

In smaller animals, such as hamsters,[8] mice, and rabbits,[3] the pregnant animal is more susceptible to endotoxin than the nonpregnant animal. In the nonpregnant rabbit, disseminated intravascular clotting could be observed after two intravenous injections of endotoxin spaced 24 hours apart, whereas the pregnant rabbit needed only one injection.[9] It was concluded from these experiments that pregnancy works as a preparatory mechanism for the generalized Shwartzman reaction in rabbits. After extensive research in the pregnant and nonpregnant rat, with glomerular capillary thrombosis used as a quantitative indicator of disseminated intravascular coagulation, it was found that the pregnant animal needed less endotoxin than the nonpregnant control (Fig. 4). It was also demonstrated in the rat model that a transferable factor was working.[11] This higher sensitization to endotoxin, however, was only partly simulated by pretreatment with estrogen or progesterone, and administration of estrogen, progesterone, or both failed to produce a significant increase of glomerular fibrin deposits and coagulation factors as seen in the pregnant animals[12] (Fig. 5). We do not know of any data which show an increase of coagulation factors after administration of estrogens in any animal, an effect known as the "pill effect" in humans.[13] The present data revealed that the pregnant minipig is also more susceptible to endotoxin infusion than the nonpregnant organism. In pregnant minipigs, the lag phase between start of the infusion and death was considerably shorter (3½ hours) than that in the nonpregnant controls (16 hours). The survival times of the pregnant and nonpregnant groups of minipigs were clearly

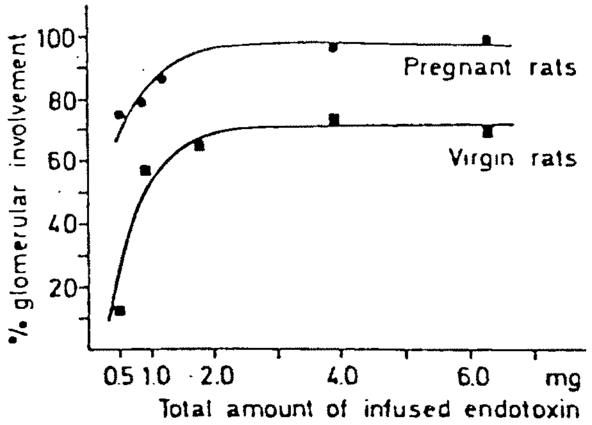

Fig. 4. Infusion of endotoxin in rats. With increasing doses and in all dose ranges the pregnant animals had more glomerular capillary thrombosis than the nonpregnant animals (*dots* represent three to five animals each).

different since the longest surviving pregnant pig died before the shortest surviving nonpregnant pig. With the Mann-Whitney U test this difference was verified. Figs. 1 and 2 also strongly suggest a difference between our two groups of animals with regard to cardiovascular and metabolic parameters, but, because of the small number of animals with different times of death resulting in "missing values" for statistical comparison, we only used descriptive statistics and did not perform an analysis of variance with repeated measures.

Although the quantitation of glomerular capillary thrombosis in these experiments awaits completion, a review of the slides reveals considerably less fibrinization of the microcirculation in the pregnant animals compared to the controls. This is of interest because, after infusion of endotoxin, the nonpregnant minipig has less glomerular capillary thrombosis than, for instance, the rat or the rabbit. Our results show that the drop in platelet number, in plasma fibrinogen concentration, and in various coagulation factors is similar in pregnant minipigs and their nonpregnant controls. However, the pregnant animals die before hemostatic breakdown is complete.

In the pregnant minipig a hypodynamic, rapid, progressive shock situation is indicated by a decrease in heart rate, cardiac output, and mean arterial pressure. Severe metabolic and respiratory acidosis is indicated by the pH and acid-base status of the pregnant animal.

It is assumed that during the last trimester of pregnancy oxygen uptake and transport are impaired through the enhanced interstitial water content which enlarges the oxygen diffusion space.[7, 13] Endotoxin induces damage of the capillary endothelium and allows for an increased extravasation of plasma water. This in turn may increase the interstitial fluid volume resulting in reduced tissue oxygenation. This is clearly indicated by our regional oxygen pressure measurements of the uterine tissue. They indicate that the pathophysiologic state after endotoxin infusion could not be compensated sufficiently by the respiratory and cardiovascular systems in the pregnant animals. This then may be the reason for the significantly earlier death.

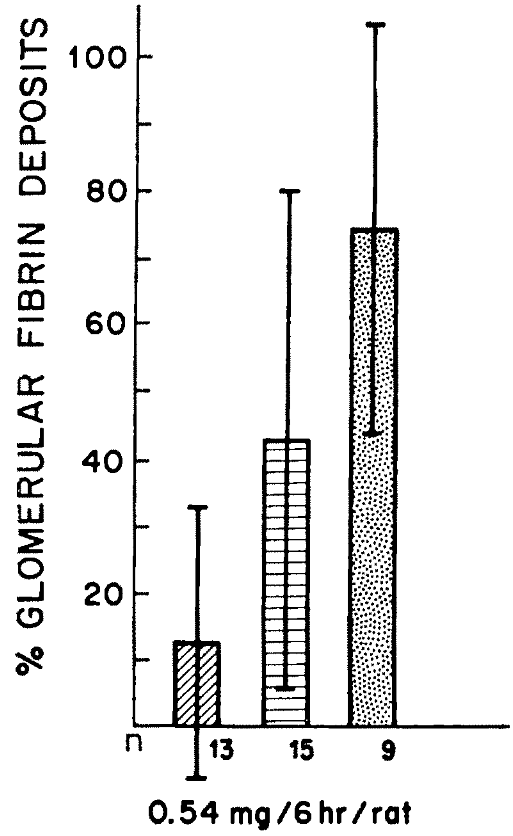

Fig. 5. Glomerular capillary thrombosis after infusion of 0.54 mg of endotoxin for 6 hours in rats. *Diagonally striated column* = Nonpregnant animals; *horizontally striated column* = estrogen and progesterone-pretreated animals; *dotted column* = pregnant animals at term (*bars* represent standard deviations). (From Nonmoff JS, Schoendorf T, Theiss W, Beller FK. Coagulation assays in pregnant rats and after pretreatment with estrogen and progesterone. J Reprod Med 1972; 9:55.)

The other two comprehensive studies[5, 6] on septicemia in larger animals are difficult to compare because single doses of endotoxin were injected. In one experiment with sheep[6] a third of the animals died between 15 and 20 minutes and seven animals were put to death 2 hours after the injection of 0.2 to 0.5 mg/kg of body weight; in another study on four pregnant baboons[5] death occurred in 24 hours in three of the four animals after injection of *E. coli* endotoxin, 5 mg/kg of body weight.

A review of the literature on experimental endotoxemia in small and larger animals reveals as a uniform result that pregnant animals of various species die with less endotoxin and/or earlier than their nonpregnant controls. Our results show that this occurs, however, for different pathophysiologic reasons: In the smaller animal the foremost event leading to death is fibrinization of the microcirculation and organ necrosis (disseminated intravascular coagulation), whereas in the minipig the leading cause of death is cardiovascular failure and metabolic breakdown.

It is not clear at what time during pregnancy this increased susceptibility starts, but there are no data yet available from animal experimentation or from experience with septic shock in humans to indicate that, in septic abortion of the first trimester, the pregnant organism serves as a "preparatory" mechanism. Based on our results, however, a start of the changes in the first trimester seems unlikely in larger animals such as the minipig, because the so-called "hypercoagulable" state, hypervolemia, and cardiovascular changes do not become apparent before the second trimester.

It is not known whether, in humans, there is a higher susceptibility to endotoxins during pregnancy as compared to the nonpregnant state. It is likely that in the human pregnancy reactions occur that are similar to those observed in the minipig, but more data are required to substantiate this concept.

We would like to thank Dr. G. Goder for his help with the statistical analysis and H. U. Spiegel for his excellent technical assistance during the experiments.

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Discussion

DR. RUDOLPH P. GALASK, Iowa City, Iowa. For a number of years Dr. Beller and his co-workers have been interested in the pathophysiologic characteristics of endotoxic shock. This article represents another contribution toward the understanding of this problem. In this presentation the various effects of endotoxin infusion in pregnant and nonpregnant minipigs were compared. Therefore, the title of this presentation is misleading, since the animals were not exposed to a septicemic episode but to the lipopolysaccharide component of *E. coli.* Regardless, the authors showed that there were profound differences when the two groups were compared. It is apparent that the pregnant animals were affected more than the nonpregnant controls by the endotoxin challenge. This supports the belief that gram-negative shock has greater consequences when it occurs in pregnant women than in nonpregnant individuals.

The authors compared the cardiopulmonary and metabolic effects of endotoxin infusion in six pregnant minipigs with those in 12 nonpregnant controls, which I assume were female animals. Unfortunately, they did not include data of untreated controls for comparison, which would have been helpful in determining if any of the effects seen were related to stress.

I must commend the authors for the intricate animal preparation and I can only hope that it did not take them as long to place their various leads, catheters, and apparatus, as it did for me to figure them out. I did have one problem with their description. In the article they describe measuring arterial pressure in the descending aorta by a catheter placed into the femoral vein. The statistical analysis of the data presented included only the means and the standard errors of the means. I would have preferred inclusion of a better estimation of the differences observed.

It is interesting that the cardiac rate increased in the control animals while it decreased approximately 50% in the pregnant animals. Further, it appears that the cardiac output increased in controls and decreased in the subjects. Also the peripheral resistance in the pregnant animal increased initially before decreasing. In the human one would expect an increase in the cardiac rate and the cardiac output and a decrease in peripheral resistance. I would like to ask Dr. Beller to discuss these apparent differences.

The data showed a marked difference when the pH and base excess were compared in the two groups. However, it appears that the pregnant animals were more acidotic then the nonpregnant controls prior to infusion of the endotoxin. Dr. Beller, how do you explain the difference in these two groups prior to endotoxin infusion?

The histograms showing Po_2 distributions in the pregnant and nonpregnant uteri prior to infusion were markedly different. In fact, the oxygen tension of the pregnant uterus before infusion was lower than that of the nonpregnant uterus at the end of perfusion. It was assumed by the authors that oxygen uptake and transport were impaired by an enhanced interstitial water content which enlarges oxygen diffusion spaces in the gravid uterus. I am not aware that this has been verified in the literature. Dr. Beller, could this simply be due to venous interference from the increased vascularity of the pregnant uterus or is there another explanation?

The data suggest that the pregnant minipig succumbs quicker than the nonpregnant animal to endotoxin, but since only a single dose was studied it remains to be determined if this animal model is more sensitive.

The authors concluded that the early deaths seen in the minipig were not associated with disseminated intravascular coagulation but rather with metabolic and cardiovascular failure. However, on review of the manuscript, it appears that the coagulation factors were consumed equally when the pregnant animals and the controls were compared. Further, data demonstrating differences in fibrin deposition in the microcirculations of the two groups were not provided.

The authors are to be commended for this undertaking and for another contribution to our understanding of endotoxin shock.

Dr. Robert Cefalo, Chapel Hill, North Carolina. There appears to be an apparent metabolic and cardiovascular difference when endotoxin is administered, whether by bolus or by a continuous infusion. With the continuous infusion the death of the animal appears to come about by deterioration of the cardiovascular system.

We have performed studies whereby we can obviate all of the cardiovascular parameters by continuous infusion, by giving indomethacin along with or prior to the continuous infusion of endotoxin.

We have also shown that many of the cardiovascular parameters were related to prostaglandin surges. Therefore, I would suggest to Dr. Beller that he look at the relationship of prostaglandins to the cardiovascular changes observed in his model.

Dr. Holzgreve (Closing). Regarding the title of our paper we have indeed studied the lipid A toxic moiety of the *E. coli* lipopolysaccharide component, but the results correspond to experimental and clinical gram-negative septicemia. We have referred many times to the differences between a bolus injection of endotoxin and a continuous infusion in an animal model and, therefore, agree with Dr. Cefalo. We believe that the infusion model resembles gram-negative endotoxemia in man more closely than a single bolus injection or two injections spaced 24 hours apart.

Of course, Dr. Galask, we have data on animals that underwent sham operation, but including them into the figures would have made the results incomprehensible. Animals that did not receive endotoxin but were operated upon and infused with saline solution did not show any change of interest.

We realized the error in regard to the arterial pressure measurements and this is corrected in the final manuscript.

For the statistical analysis of our data we used descriptive methods and calculated the means and the standard errors of the means. Because of the high mortality in the pregnant group of animals statistical analysis was available only for 3 hours. The estimation of the observed differences was performed by the Wilcoxon test and the calculations by the data-processing program of the Statistical Package for the Social Sciences. Only higher numbers of animals in both groups would allow us to give a better estimation of the differences observed.

Dr. Galask's reference to human septic shock regarding a hyperdynamic reaction indicated by an increase in the cardiac rate and cardiac output can be seen in nonpregnant animals such as the baboon and the minipig. In the pregnant animals we unexpectedly observed a low–cardiac output shock syndrome with a decreased central venous pressure, tachycardia, and hypotension.

The differences between pH and base-excess data between the pregnant animals and their nonpregnant controls became obvious very early in the experiment. This can be attributed to the dramatic increase in lactate levels, which is due to decreased oxygen consumption as a result of endotoxin on the cell membrane and the shift from oxidative phosphorylation to anaerobic glycolysis. Romanovsky et al.[1] pointed out that skeletal muscle is the main lactate-releasing organ during endotoxin shock; the increased lactate cannot be metabolized by the liver and kidney, which have a severely affected microcirculation,[2] as also indicated by the observed rapid onset of anuria in our pregnant group of animals.

Dr. Galask pointed to the differences in the histograms between the pregnant and nonpregnant minipigs before infusion. This may very well be due in part to venous interference from the increased vascularity of the pregnant uterus. There are no similar data available on pregnant animals. Important for interpretation of the histograms is the marked shift to the left in Po_2 of the pregnant animals soon after the start of the endotoxin infusion, indicating a dramatic increase in anoxic measurements as opposed to the unchanged histograms in the nonpregnant minipigs during the same period of time.

The decrease in clotting factors indicates an equal consumption after endotoxin administration in pregnant animals as compared to the controls. The pregnant minipigs, however, were dead before concentrations of plasma fibrinogen and other factors, for instance, factor XII, were in a range of consumptive coagulopathy. In this respect our results are in agreement with the study by Hammon et al.[3] They concluded, from their experiments with live *E. coli* injections into primates with and without arvin pretreatment, that their results do "not support an obligatory role for intravascular coagulation or fibrin formation in primate sepsis . . . . Vascular damage and malfunction, secondary to mediators released by platelets, leukocytes, red cells or Hageman factor, are not ruled out."

Dr. Cefalo, as you have shown, prostaglandins are obviously involved in this reaction, but this is on our list for further experiments.

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Plasma vitamin C and uterine cervical dysplasia

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Bronx, New York

Plasma concentrations of vitamin C were determined in a case-control study of women (n = 80) who had sought a Papanicolaou test in the Bronx Municipal Hospital Center. Controls (n = 34) were women having negative cytologic tests, negative colposcopic findings, and no known gynecologic dysfunction. Cases (n = 46) were defined as women who had either one positive or two consecutive suspicious Papanicolaou smears in a 12-month period. The mean concentration of vitamin C in the plasma was significantly lower in the cases than in the controls (0.36 versus 0.75 mg/dl, $p < 0.0001$). Cases were further stratified according to the histopathologic diagnosis. The data direct attention to a possible etiologic association of vitamin C in human cervical epithelial abnormalities. A clinical trial with vitamin C intervention is suggested. (Am J Obstet Gynecol 1985;151:976-80.)

Key words: Cervical dysplasia, plasma vitamin C, cytology, colposcopy

The role of nutrition in human cancers is of current interest. We previously reported a case-control dietary survey in which we asked the question whether a deficiency in the dietary intake of vitamin A could be a significant factor in the precursor lesions of the human uterine cervix.

There was a large variability in the dietary intake of vitamin A among women with mild and moderate dysplasias which precluded statistical significance. However, among the subset of women diagnosed histopathologically as having severe dysplasia or carcinoma in situ, a low dietary intake of vitamin A was detected compared to that in the matched control group, according to the analysis of 3-day food records.[1, 2]

An unexpected finding in that study was a statistically significant low dietary intake of vitamin C in all the cases no matter how they were stratified. Multivariate analysis, controlling for other confounding variables known to be important epidemiologic factors in cervical cancer, such as early onset of sexual activity, multiple partners, and multiple pregnancy, established vitamin C as an independent risk factor in all degrees of dysplasia and carcinoma in situ. This relationship held in both lower and higher socioeconomic groups.[3]

As a follow-up study to that finding, we have investigated the plasma concentrations of ascorbic acid in another group of women requesting Papanicolaou smears. Here, we report a significant decrease in the plasma ascorbic acid concentrations of women having cervical lesions compared to those of controls.

Methods and material

Selection of subjects. The study group (both controls and cases) were recruited from a random group of women who obtained a Papanicolaou smear in a cytology screening clinic at the Bronx Municipal Hospital Center, Bronx, New York, during a period of 1 year. The Papanicolaou smear finding was the screening basis for identifying controls versus cases. All women were additionally examined by colposcopy when a repeat Papanicolaou smear was performed.

Control subjects were further screened as the subset of women who by history were free of any prior gynecologic disease or known dysfunction and had negative Papanicolaou smears, a negative colposcopic examination with clear delineation of the squamocolumnar junction of the cervix, and a normal pelvic examination.

Cases were defined as women who had either two consecutive suspicious (Class III) or one positive Papanicolaou smear (Class IV) in the 12-month period. A Class III smear is usually indicative of dysplastic changes; a Class IV smear may be correlated with dysplasia or carcinoma in situ. When colposcopy was routinely performed biopsy of any abnormal site was performed.

All cytologic slides were interpreted by the staff cytopathologists at Bronx Municipal Hospital Center.

Our previous dietary study showed that younger age, socioeconomic status, and parity are important risk fac-

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tors for cervical dysplasias, confirming similar findings in other studies.[3-5] In the present study, we did not carry out any dietary survey nor did we match cases and controls in terms of any independent variables such as age, parity, sexual activity, or socioeconomic status. However, in the recruitment of the controls and cases, all were drawn from the same catchment area. The women who apply to the Bronx Municipal Hospital Center for their health care have similar socioeconomic backgrounds and approximate a fairly homogeneous population group. Informed consent was obtained from each participant.

Determination of vitamin C in blood. At the time of enrollment when the Papanicolaou smear was obtained, a peripheral venous blood sample was collected from each study subject in heparin-coated tubes. Plasma was separated by centrifugation at 800 × g for 15 minutes. Vitamin C levels in the plasma were determined within a few hours of its collection according to the method of Zannoni et al.[6]

Plasma was deproteinized with 40% trichloroacetic acid. The acidified plasma was allowed to stand in ice for 10 minutes to ensure completion of precipitation and then centrifuged at 1,500 × g for 15 minutes. The supernatant was incubated with 85% o-phosphoric acid, 1% α,α'-dipyridyl, and 3% ferric chloride successively for 15 minutes. The color was evaluated at 525 nm. If the last addition of reagent in plasma samples resulted in turbidity, the tubes were centrifuged at 1,500 × g for 5 minutes. Vitamin C content is expressed as milligrams per deciliter of plasma.

Statistical analysis of the plasma values. The data were statistically treated as follows. Mean blood levels of vitamin C of all cases and controls were compared with Student's t test. The cases were stratified by histopathologic subgroups as (1) inflammatory conditions, for instance, cervicitis; (2) mild and moderate dysplasias; and (3) severe dysplasia and carcinoma in situ. A one-way analysis of variance was used to test for any statistical differences among the mean values of the subgroups.

Duncan's multiple range test was used for post hoc comparisons.

Results

Case-control subjects. Of the pool of 130 women we approached who were seeking a Papanicolaou test in the year of this study (1982 to 1983), 20 were ineligible because an initial suspicious Papanicolaou smear could not be reconfirmed and colposcopic examination was considered normal. Of 56 potential control subjects and 54 potential cases, 34 and 46, respectively, were willing to be recruited and volunteered to undergo collection of peripheral venous blood samples.

The age distribution among control and case subjects is presented in Table I. Among the cases, the highest number of subjects was in the 25- to 34-year-old group. The controls were fairly evenly distributed between ages 25 and 55.

Table I. Age distribution in the case-control subjects

	Percent of total	
Age (yr)	*Control (n = 34)*	*Case (n = 46)*
18-24	15	24
25-34	27	39
35-44	32	22
45-55	26	15

Table II. Histopathologic diagnosis among cases (n = 46) with abnormal Papanicolau smears

Histopathologic diagnosis	*No.*	*%*
Cervicitis	15	33
Mild and moderate dysplasia	14	30
Severe dysplasia and carcinoma in situ	17	37

Colposcopic and cytopathologic results. The cytopathologic diagnoses of the colposcopically directed biopsies among the 46 cases are listed in Table II. In 15 subjects inflammatory changes were diagnosed as cervicitis; 14 of the cases had the histopathologic changes of mild and moderate dysplasia and 17 of the biopsies were considered severe dysplasia or carcinoma in situ.

Plasma levels of vitamin C. The overall mean plasma vitamin C levels for the controls and cases are seen in Table III. Significantly lower plasma levels of ascorbic acid were detected in the cases compared to the controls ($p < 0.0001$). When the cases were further stratified by histopathologic diagnoses, the analysis of variance for the mean differences in plasma ascorbic acid levels among the various cervical epithelial lesions was highly significant (Table IV). The controls had the highest values and the cases with inflammatory conditions the lowest.

Comment

The importance and role of diet and nutrition in the multifactorial etiology and possibly as a public health measure in the prevention of human cancer are of current interest. It has been estimated that as many as 60% of cancers in women and 40% in men are directly influenced by diet and nutrition.[7] There is a growing investigative concern with specific nutrients, particularly the poorly understood roles of vitamins and minerals. In general terms, essential nutritional-nurture factors have long been known to be important for normal reproduction, growth, and development. In the

Table III. Plasma vitamin C levels in case-control subjects

Population	*n*	*Mean ± SD (mg/dl)*	*p Value*
Control	34	0.75 ± 0.33	<0.0001
Case	46	0.36 ± 0.22	

case of vitamin A, while its mechanism of action is obscure, a role in the maturation, differentiation, and maintenance of epithelial structures has long been recognized.

In the present study, a statistically significant decrease in plasma concentrations of vitamin C has been detected in asymptomatic women having dysplastic epithelial cell anomalies of varying degrees of the cervix. This finding is additionally significant because it is consistent with our previous report of a low or deficient dietary intake of vitamin C in women having dysplasias or carcinoma in situ. It is interesting to note that in the dietary survey the low intake of vitamin C was greatest among women having the more advanced severe dysplasia and carcinoma in situ.[3] In contrast, in the present study, the decrease in plasma concentration was greatest in the women with inflammatory histopathology (Table IV). The explanation of these differences in dietary versus plasma concentrations in the various epithelial atypias is not clear. Differences in the nutrient requirement and metabolism of vitamin C in an inflammatory response as opposed to preneoplastic changes in cervical epithelia require investigation.

In examining vitamin C's biochemical roles, questions arise as to how the nutrient may influence epithelial carcinogenesis. The ability of ascorbic acid to serve as an electron donor may potentiate its role in maintaining normal epithelium. This donor capability may alter the chemical properties of vitamin C and establish a potential electron acceptor role for oxidized ascorbic acid. Ascorbate's ability to both receive and donate electrons may endow it with a potential to enhance, inhibit, or modulate the activity of other nutrients as well as anticancer agents.

Pelleter[8] has shown that there is a decreased excretion of vitamin C in people who smoke, which may be indicative of an increased vitamin C requirement in smokers. Leuchtenberger and Leuchtenberger[9] reported that the addition of vitamin C can limit the carcinogenic effect of tobacco and marijuana in hamster lung cultures by preventing abnormalities in cell division. It has been suggested that vitamin C may lessen the carcinogenic effect of cigarette smoking as a risk factor in lung cancer.[8]

Of more direct relevance is a report of ascorbic acid content in uterine cervical neoplasms. Using a microspectrophotometric technique, Soviet investigators[10] reported decreased levels of ascorbic acid in malignant lesions and correlated ascorbic acid content with the degree of differentiation and the histogenesis of the neoplasm. Their data revealed vitamin C depletion in precursor lesions and increased content of ascorbic acid in the tumor per se.[10] This finding is in agreement with a theory of encapsulation which postulates a proliferation of collagen (associated with elevated ascorbic acid) as part of a mechanism to control the invasive potential of the neoplasm.[11]

Table IV. Comparative mean plasma vitamin C levels among cases stratified by histopathologic diagnosis

Histopathologic diagnosis	*n*	*Mean ± SD* (mg/dl)*
Control	34	0.75 ± 0.33
Cervicitis	15	0.25 ± 0.12
Mild and moderate dysplasia	14	0.34 ± 0.15
Severe dysplasia and carcinoma in situ	17	0.48 ± 0.28

*Analysis of variance: $p < 0.0001$.

The findings in this study additionally emphasize the importance of nutrient interactions as significant etiologic factors in uterine cervical disease. Further attention should be directed to eating habits, food preparation and consumption, and life-style as having an important bearing on sound nutrition. The study also serves to direct attention to opportunities to gain further insight into hypothetical mechanisms of action of the essential nutrients as biomodifiers influencing epithelial structure and function. In this latter connection, nutrients used as pharmacologic agents in intervention clinical trials in blinded, placebo-controlled studies should be investigated. The importance of any single nutrient, however essential, depends on its cellular interaction. Clearly, sound nutrition involves an interaction of multiple nutrients and their metabolites. The present findings are important to any further intervention clinical trials to evaluate the role and efficacy of vitamin C in treating precursor lesions and in probing the conceptual validity of an active program in cervical cancer prevention. While some of these lesions may spontaneously regress, clarification of the physiologic role of essential nutrients such as vitamins A, C, and E or folate and their interactions with each other or their pharmacologic supplementation as public health measures to accelerate the regression of precancerous lesions requires further investigation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Michael Newton, Chicago, Illinois. Dr. Romney's article prompted me to reflect on the various theories of the cause of cervical cancer expounded over the past 30 years or more. I remember being excited in the mid-1950s by the concept that human smegma was important. As it turned out this idea may not have been too far off the mark. Then came the epidemiologic phase, when early and multiple coitus was found to be related to invasive and later to intraepithelial cancer of the cervix. At this point, I was diverted by recalling the circumcision debate. The originally postulated connection of circumcision with cervical cancer dissolved, at least in part, when it was found that neither wives nor husbands nor even physicians could accurately determine whether a man was circumcised. This negative observation has been somewhat revised recently by the concept of the high-risk male. Then we have had the virus phase in which first herpesvirus type 2 and currently human papillomavirus has been implicated. Of recent interest, too, has been the possible connection of diet with the development of the disease, a field which Dr. Romney and his associates have been diligently cultivating.

With so many possible causes of cancer of the cervix, it is apparent that we have not reached the true one. One of the problems is exemplified by a sentence in the introduction to Dr. Romney's article. In discussing data previously reported by his group on dietary intake and cervical intraepithelial neoplasia he stated: "A low intake of vitamin C *resulted* in a substantially higher estimate of relative risk of cervical dysplasia." But this is a relationship and not necessarily cause and effect.

I have two minor comments about the article. First, the statistical measure entitled "Duncan's multiple range" was quite beyond me and I found the explanation in Table IV to be unsatisfactory. I am sure that most of you nonstatisticians would have the same difficulty. Second, in this day and age, I think that Papanicolaou smears should be classified by the cytologic appearance of the cells, rather than as suspicious or positive.

I have three questions about the article itself.

First, how were the control patients selected and why were only 34 of the 56 controls (61%) and 46 of the 54 cases (85%) finally included?

Second, Dr. Romney's results show that the vitamin C levels in the cases were significantly lower than those in the controls. Assuming the accuracy of the methods used, I still wonder if these levels are related to recent ingestion of vitamin C or to sustained low dietary intake of this nutrient. Does Dr. Romney have any information on this subject? It would have been nice to have a dietary history on the patients studied.

Third, why were vitamin C levels higher in severe than in moderate dysplasia and lowest in inflammatory conditions?

How do Dr. Romney's observations fit into the picture of cervical cancer and its cause? Most recent information on the place of human papillomavirus in the development of cervical cancer indicates that the koilocytic and other changes in the epithelial cells may persist or regress but that certain other factors may determine the development of neoplasia in a cervix that is already affected by the virus. Perhaps vitamin C contributes, as Dr. Romney suggests, to the immunologic defense against cancer, although this function would seem to be more relevant to the onset of invasion rather than to changes within the epithelium.

Speculation aside, I think that Dr. Romney and his group deserve credit for tackling a very difficult subject in a scientific way. I hope that they will soon progress to prospective studies. As yet I am not convinced that vitamin C is an essential part of the cervical cancer story, nor would I yet recommend that my patients take vitamin C to prevent cervical intraepithelial neoplasia. However, I shall cling to my prejudiced belief that a good diet including vitamin C is a protective measure against illness and to the dogma that high doses of vitamin C do in a mysterious way sometimes prevent and frequently ameliorate the symptoms of the common cold or "coldus vulgaris."

Dr. Kamran S. Moghissi, Detroit, Michigan. My comment is that dietary recall extending beyond 24 hours is extremely hazardous, and I think data based on this kind of information can be misleading.

Has Dr. Romney tried to look at the vitamin C story relative to cancer at other sites such as vulva, larynx, or skin to see whether the tissue vitamin C concentration might be altered at these other sites, because this may be simply the property of this particular vitamin at the cancer site rather than a cause-and-effect relationship.

Dr. Romney (Closing). We have not carried out a specific dietary survey on the group of patients reported on in this study in contrast to the initial dietary survey that unexpectedly revealed a vitamin C deficiency in the dietary intake of women with dysplasias.

Dietary intake studies are difficult and expensive. There is the very real criticism that they are "soft" science because it is difficult for anyone to recall with great accuracy the quality and quantity of food consumed with each meal.

On the other hand, in the hands of technically expert people, nutritionists who can instruct patients with food models to quantity and quality of food and then interpret data obtained into a coding system which can be processed for computer analysis, the data that can provide directions for investigation begin to emerge. It was such a consideration that prompted our group into looking at plasma concentrations of vitamin C.

Dr. Newton touched on what is still a very confused question concerning the primary etiology of cervical cancer. It continues to be confused, with a great deal of current interest in papillomavirus. What can be said at this time is that in the early lesions of mild or moderate dysplasia there is a significant spontaneous regression rate. We believe that a placebo-controlled clinical trial carried out carefully with quality control over a significant period of time with a large enough population group can evaluate whether any of these nutrients used pharmacologically have a role to play in cancer chemoprevention.

One of the end points being looked at in a study sponsored by the National Institutes of Health is whether in actual fact retinoids and/or vitamin C might have an influence in enhancing the immune response in relation to early papillomavirus infections.

In answer to Dr. Newton's question, all the patients selected were volunteers with informed consent. The discrepancies in some of the numbers were related to the fact that some patients did not wish to participate.

With respect to Dr. Moghissi's question, we decided a long time ago that we were going to stay focused on the cervix. While there is a great temptation to look at other genital tract malignancies, we have determined to stay with the cervix with the hope that if we can put something together there, maybe we can look at some other neoplasms. In connection with the cervix, one of the interesting developments has been the success in our laboratory in measuring tissue concentrations of vitamin C. The question has been repeatedly raised whether there is any obvious relationship between plasma concentration and tissue concentration. We are looking into this aspect at this time.

Predictive value of CA 125 antigen levels in second-look procedures for ovarian cancer

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Serum CA 125 levels were evaluated in 44 patients undergoing 56 second-look or subsequent laparoscopies (43) and laparotomies (13) for ovarian cancer. In each patient studied, a previous CA 125 level had been ≥35 U/ml. Clinical or radiologic evidence of tumor was absent in all patients at the time of surgical evaluation. CA 125 levels were <35 U/ml in 36 cases (64%); 14 patients were free of tumor, while 22 were found to have tumor at surgery. CA 125 levels were ≥35 U/ml in 20 cases; 18 had tumor at surgery, one has had recurrence of tumor, and the other remains clinically free of tumor at 3 months. A CA 125 level <35 U/ml was not predictive of the presence of intraperitoneal tumor; however, when tumor was present in this group of patients, the largest tumor mass did not exceed 1 cm. In contrast, a CA 125 level ≥35 U/ml was a strong predictor of the presence of intraperitoneal tumor or future recurrence. These data suggest that second-look procedures may not be required in the select group of patients with CA 125 levels ≥35 U/ml. (AM J OBSTET GYNECOL 1985;151:981-6.)

Key words: CA 125 antigen levels, ovarian cancer, second-look operation

In many institutions second-look surgical procedures (laparotomy or laparoscopy) have become an integral part of the treatment plan for ovarian cancer.[1-5] Such procedures are employed to determine subclinical tumor status and thus indicate whether treatment should be instituted, modified, or discontinued. Findings at second-look procedures are also of prognostic significance, since survival rates are higher among patients who are free of tumor compared with patients with persistent disease.[5]

CA 125 is an antigenic determinant associated with a mucin-like glycoprotein that has a molecular weight exceeding 500,000 daltons. This determinant is recognized by the murine monoclonal antibody OC 125, which reacts with over 80% of epithelial ovarian cancers.[6] A radioimmunoassay has been developed to detect CA 125 in serum.[7] When this assay was used, only 1% of 888 apparently healthy individuals and 6% of 143 patients with nonmalignant diseases had serum CA 125 levels ≥35 U/ml. In contrast, in 82% of 101 patients with ovarian cancer the antigen levels were ≥35 U/ml.[8] When 38 patients were monitored serially, CA 125 levels correlated with disease progression or regression in 93% of instances.[8]

The purpose of this study was to determine the predictive value of CA 125 levels at the time of second-look surgical procedures. Although the introduction of laparoscopy has reduced the number of laparotomies performed, this invasive monitoring technique is still associated with significant morbidity.[3, 5] Therefore a serum marker that could be used to identify patients with subclinical intraperitoneal tumor would clearly be advantageous.

Material and methods

Forty-four patients with epithelial ovarian carcinoma underwent 35 second-look procedures (28 laparoscopies, seven laparotomies) and 21 third-look and subsequent procedures (15 laparoscopies, six laparotomies). A second-look procedure was defined as a surgical evaluation after an initial period of observation (two cases) or after completion of the first course of therapy after primary surgery (chemotherapy in 31 cases and radiation therapy in two cases). All other procedures were classified as third-look or subsequent. No evidence of disease was detected on physical examination or on chest roentgenogram in any patient. Patients found to have evidence of tumor on computed tomography were excluded.

The median age of the study population was 52 (range, 22 to 79). Ovarian cancer was classified as Stage I in one patient, Stage II in two patients, Stage III in 34 patients, and Stage IV in seven patients. Tumors were classified as borderline in two cases, grade 1 in three cases, grade 2 in nine cases, and grade 3 in 30 cases. Second-look procedures were performed an average of 8 months (range, 3 to 12) after initial diagnosis

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Table I. CA 125 levels and surgical findings at second-look procedures

Finding	*n*
CA 125 <35 U/ml	
No evidence of disease	9
7 laparoscopies	
2 laparotomies	
↓	
No recurrences	
Intraperitoneal tumor present	18
15 laparoscopies	
3 laparotomies	
Total	27
CA 125 ≥35 U/ml	
No evidence of disease	1
Unsatisfactory laparotomy	
↓	
Recurrence at 2 months	
Intraperitoneal tumor present	7
6 laparoscopies	
1 laparotomy	
Total	8

and surgery. Third-look and subsequent procedures took place from 9 to 70 months (mean, 24 months) after primary surgery. Positive cell washings or histologically positive biopsies were considered evidence for persistent or recurrent tumor.

The immunoradiometric assay to detect CA 125 in peripheral blood was performed as has been described elsewhere.[7, 8] CA 125 present in 100 μl aliquots of serum is bound by purified OC 125 on a solid-phase immunoadsorbent. Since more than one CA 125 site is associated with each antigen molecule, bound antigen is detected by incubation with iodine 125–labeled OC 125, and CA 125 levels are expressed in arbitrary units based on comparison with primary reference standard. Technicians who performed the CA 125 assays had no knowledge of the population being studied. Each patient had to have had at least one elevated serum CA 125 level, which was defined, based on prior studies, as ≥35 U/ml.[7, 8] This study was perfomed retrospectively. Patients were included if a serum CA 125 level had been obtained within 1 month of the corresponding surgical procedure. Thirty-eight samples (68%) were obtained within 1 week of operation.

The statistical significance of the correlation between tumor size and CA 125 level was evaluated by means of χ^2 analysis.

Results

Second-look procedures. Twenty-seven (77%) of the 35 second-look procedures were associated with normal CA 125 levels (<35 U/ml) while eight (23%) had elevated levels (Table I). No tumor was found in nine (33%) of the 27 patients with normal CA 125 levels (seven laparoscopies, two laparotomies), and none of these nine patients had recurrent disease during a median follow-up period of 10 months (range, 2 to 61 months). In the remaining 18 patients with normal antigen levels, tumor was demonstrated in 15 laparoscopies and three laparotomies.

Eight patients had elevated antigen levels ranging from 35 to 545 U/ml (mean, 189 U/ml). Among these patients with elevated CA 125 levels, tumor was present at seven of the eight procedures (six laparoscopies, one laparotomy). Washings from one of these patients demonstrated "degenerating possible tumor cells," and tumor subsequently recurred. The single patient with an elevated CA 125 level in whom tumor was not demonstrated at surgery suffered a recurrence 2 months after a laparotomy that was considered to be inadequate because of adhesions. Thus no patient with an elevated antigen level remained free of disease.

No patient with a CA 125 level <35 U/ml had a tumor mass exceeding 1 cm in diameter. Twenty-five percent of eight patients with CA 125 levels ≥35 U/ml had tumor masses that exceeded 1 cm in diameter at second-look surgery, whereas none of 27 patients with CA 125 levels <35 U/ml had tumor masses greater than 1 cm (Table II).

Third-look and subsequent procedures. Nine (43%) of 21 patients undergoing third-look or subsequent procedures had serum CA 125 levels <35 U/ml (Table III). Among these nine patients, no tumor was found in five cases: four laparoscopies and one laparotomy. Four remain clinically free of disease (three at 1 month and one at 8 months); in the other, tumor recurred clinically 11 months after a laparoscopy. Four months after this procedure, the CA 125 level rose to 121 U/ml, thereby becoming elevated 7 months before disease recurred clinically.

Elevated antigen levels ranging from 35 to 980 U/ml (mean, 318 U/ml) were found in 12 patients. Intraperitoneal tumor was present in 11 of these 12 patients (92%) (six laparoscopies, five laparotomies). The other patient whose antigen level was elevated underwent laparoscopy with a negative result and remains clinically free of disease after 3 months of follow-up.

None of nine patients with CA 125 levels <35 U/ml had tumor masses >1 cm, whereas eight of 12 patients (67%) with levels ≥35 U/ml had tumor masses >1 cm (Table II).

Comment

Earlier studies have suggested that the CA 125 radioimmunoassay is useful for monitoring disease status in patients with epithelial ovarian cancer.[8] This antigen has also been detected in the serum of patients with nonovarian gynecologic malignancies, indicating that these neoplasms may also be amenable to monitoring by this technique.[9] Because of the low level of false positive elevations of CA 125 observed in patients with benign gynecologic disease, this assay warrants further

Table II. CA 125 levels and tumor size found in patients at second-look and subsequent procedures

Tumor size (cm)	*CA 125 levels in second-look procedures*				*CA 125 levels in subsequent procedures*			
	<35 U/ml		*≥35 U/ml*		*<35 U/ml*		*≥35 U/ml*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
No tumor	9		1		5		1	
≤0.4	13		4		4		0	
0.5 to 1.0	5		1		0		3	
>1.0	0	0*	2	25*	0	0	8	67
Total	27		8		9		12	

*$\chi^2 = 3.271$, $0.05 < p < 0.07$.

Table III. CA 125 levels and surgical findings at third-look and subsequent procedures

Finding	*n*
CA 125 <35 U/ml	
No evidence of disease	5
4 laparoscopies	
1 laparotomy	
↓	
1 recurrence	
Intraperitoneal tumor present	4
4 laparoscopies	
Total	9
CA 125 ≥35 U/ml	
No evidence of disease	1
1 laparoscopy	
↓	
3-month follow-up	
Intraperitoneal tumor present	11
6 laparoscopies	
5 laparotomies	
Total	12

assessment as a means for early detection of ovarian cancer.[10] In this study we examined the predictive and prognostic value of the CA 125 assay at the time of second-look and subsequent evaluative surgery for ovarian cancer.

Second-look procedures in ovarian cancer are being performed more frequently because of the favorable survival rates associated with a complete response to therapy.[11, 12] With these procedures one can assess tumor status and modify treatment plans accordingly. When it is possible, prolonged treatment with alkylating agents should be avoided.[13] Although somewhat ameliorated by the introduction of laparoscopy, significant morbidity and prolonged hospital stay are associated with these procedures.[3, 5]

In our study, serum levels of CA 125 reliably indicated the presence of intraperitoneal tumor and may therefore provide an alternative means of detecting persistent ovarian cancer. None of the patients in this study had evidence of tumor on physical examination or radiographic evaluation. Although this study population included only those patients known to have previously elevated serum CA 125 levels, at least 82% of all patients with ovarian cancer will be found to have elevated CA 125 levels on assay.[8]

A CA 125 level <35 U/ml was not predictive of intraperitoneal tumor status; however, among second-look procedures, patients with both a negative antigen level and negative findings at surgery have experienced no recurrence of tumor over a median follow-up of 10 months. Conversely, an elevated antigen level was found to be a strong predictor of intraperitoneal disease. For both second-look and subsequent procedures, intraperitoneal tumor was present at surgery or recurred early in 19 of 20 patients with elevated CA 125 levels (≥35 U/ml). In the one patient in whom no disease was found, only 3 months have elapsed since a third-look laparoscopy was performed (Table I).

The sensitivity, specificity, and predictive value of the CA 125 assay for determining the presence of intraperitoneal tumor at the time of second-look or subsequent procedures for ovarian carcinoma are presented in Tables IV and V. In Table IV the end point is defined as surgically detectable tumor. In Table V the patients with <6 months of follow-up have been excluded, and the end point is redefined as the detection of tumor at surgery or future recurrence. In Table IV, sensitivity is defined as the proportion of patients with intraperitoneal tumor detected at surgery who have CA 125 levels ≥35 U/ml. Specificity is the proportion of patients who are free of tumor who have CA 125 levels <35 U/ml. The predictive value of a positive CA 125 assay is the proportion of patients with CA 125 levels ≥35 U/ml in whom intraperitoneal tumor was actually found at surgery. In Table V, these same three parameters have been calculated with the end point redefined to include future recurrence as well.

Examination of these tables reveals that, although the assay offers a moderate degree of sensitivity, the specificity and predictive values are very high, reaching 1.0 for the prediction of intraperitoneal tumor or future recurrence. Thus a patient with a CA 125 level <35 U/ml may or may not be free of tumor. On the other hand, all patients with CA 125 levels ≥35 U/ml will

Table IV. Parameters of CA 125 assay*: prediction of tumor status at surgery

	Second-look procedures (n = 35)	*Third-look and subsequent procedures (n = 21)*	*All procedures (n = 56)*
Sensitivity	0.28	0.73	0.45
Specificity	0.90	0.83	0.88
Predictive value of positive test	0.88	0.92	0.90

*Positive test = CA 125 ≥35 U/ml.

Table V. Parameters of CA 125 assay*: prediction of tumor status at surgery or of future recurrence†

	Second-look procedures (n = 31)	*Third-look and subsequent procedures (n = 17)*	*All procedures (n = 48)*
Sensitivity	0.31	0.69	0.45
Specificity	1.00	1.00	1.00
Predictive value of positive test	1.00	1.00	1.00

*Positive test = CA 125 ≥35 U/ml.
†Minimum of 6 months of follow-up.

have persistent intraperitoneal tumor. Therefore, unless secondary cytoreductive surgery is being contemplated, surgery to determine the need for further therapy may be obviated in this latter group.

Previous studies have indicated that only 1% of apparently normal individuals have serum CA 125 levels ≥35 U/ml.[8] However, inasmuch as the CA 125 antigen is found in tissues of müllerian duct derivation[6] and since most patients undergoing second-look procedures for ovarian cancer will have previously had the reproductive organs extirpated, one possible source of serum antigen is not present in this population.[10] Furthermore, among the 20 patients with elevated antigen levels in the present study, the mean was 266 U/ml and only three patients (15%) had levels <65 U/ml. In previous studies CA 125 levels were >65 U/ml in only 0.2% of normal blood donors, corresponding to a minimum specificity for ovarian cancer at this level of 0.998.[7] These data increase one's confidence that an elevated CA 125 level obtained at the time of a second-look procedure is truly attributable to ovarian cancer. Modifying the interval from initial surgery to the second-look procedure might improve the predictive power of a negative result on antigen assay.

Serum antigen levels were found to correlate with the size of tumor found at second-look or subsequent operations (Table II). In the 27 second-look procedures associated with CA 125 levels <35 U/ml, none of the tumor masses present exceeded 1 cm in diameter. Similar data obtained from third-look and subsequent procedures further validate this observation. Absence of an elevated antigen level reliably indicates that, if intraperitoneal tumor is present, the largest tumor mass will probably not be >1 cm.

Ovarian cancer spreads on peritoneal surfaces and is difficult to detect clinically. Survival rates among patients with this neoplasm have improved with improved responses to combination chemotherapy. Nevertheless, repeated laparoscopies or laparotomies to assess therapeutic response are associated with morbidity. This study indicates that a CA 125 level <35 U/ml does not confirm that the patient has responded completely to treatment; however, if tumor was present, the largest mass did not exceed 1 cm. On the other hand, an elevated antigen level is nearly always associated with the presence of intraperitoneal tumor or future recurrence. These results suggest that, unless cytoreductive surgery is being contemplated, second-look procedures may not be necessary in the select group of patients who have been treated for ovarian cancer but who are found to have elevated levels of CA 125 on follow-up.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Hervy E. Averette, Miami, Florida. In October, 1983, the first clinical paper on the use of an immunoradiometric assay of the epithelial ovarian tumor antigen CA 125 was published by the same investigators from Boston in *The New England Journal of Medicine.*[1] Significant findings included: (1) 82% of the 101 patients with ovarian carcinoma had elevated antigen in serum (>35 U/ml); (2) only 6% of 143 patients with benign disease had significant levels; and (3) 28% of 200 patients with nongynecologic cancer had levels >35 U/ml. It was concluded that CA 125 may be a generally useful marker for monitoring disease in patients with epithelial ovarian cancer. I had the opportunity to review this report prior to publication and I was favorably impressed with the findings.

In a subsequent article, elevated levels of CA 125 (>35 U/ml) were found in all of four patients with fallopian tube carcinoma, in approximately 48% of patients with endometrial adenocarcinoma, and in 83% of patients with adenocarcinoma of the endocervix. Of the patients with endometrial carcinoma, elevated CA 125 levels were found in 77% of those with Stage IV or recurrent disease. Unfortunately, none of the patients with Stage I or Stage II disease were found to have elevated CA 125 levels.[2]

Today's article extends the conclusions from the first and focuses on the possibility that CA 125 levels may predict whether an invasive second-look procedure is required after treatment for epithelial ovarian cancer. Observations were made on a select group of 44 patients with elevated CA 125 levels prior to treatment who had no clinical evidence of cancer after treatment. All eight patients with elevated CA 125 ultimately had cancer, which to me is a significant finding. However, the authors state: "No patient with a CA 125 level <35 U/ml had a tumor mass exceeding 1 cm in diameter." Of the 18 patients in this group, 15 were evaluated by laparoscopy only, while three had laparotomy. Also, among the entire series, there were 56 surgical procedures to determine extent of disease and 43 were by laparoscopy and 13 by laparotomy.

The major problem I have with what is otherwise concise and clearly presented data is the surgical method by which extent of disease was determined. At this time, most gynecologic oncologists that I know who continue to use laparoscopy for an initial second-look procedure consider negative findings inconclusive and proceed to laparotomy. Therefore, I would like to ask the following: (1) Do the authors feel that negative laparoscopy is sufficient to conclude that the patient is free of disease without laparotomy? (2) If minimal disease (<1 cm) is noted at laparoscopy, is this procedure alone sufficient to exclude larger tumor masses beneath the diaphragm or in the retroperitoneal spaces?

This may be a minor point, but since all conclusions are based on extent of disease found at second and subsequent surgical procedures, comments by the authors may clarify some questions that others may raise.

I might add that we have had limited experience with CA 125 in patients with epithelial ovarian tumors. When it was recently advertised by a local reference laboratory that the test was available, it was used in three patients prior to and after surgery. In each patient, serum CA 125 correlated with what would be expected from the authors' data. Cost to the patient from the reference laboratory was $50.00. Two weeks ago, we obtained the CA 125 kit from Abbott Laboratories for our own laboratory, which permits one to perform 100 assays at the cost of <$500.00 per kit, or $5.00 per test. The only other major requirement for the assay is availability of a γ-counter. Three patients with advanced epithelial ovarian cancer had elevated CA 125 levels and two with minimal disease had normal values. We anticipate that within a short period of time we will be able to make observations similar to those reported today and hopefully confirm the value of this tumor marker in the management of our patients.

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Dr. George Mitchell, San Antonio, Texas. I would like to take a look "down memory lane" at the long search for a marker for ovarian carcinoma. I recall that years ago the enzyme β-glucuronidase was given extensive clinical trials. Since then we have been occupied with carcinoembryonic antigen, of which CA 125 seems to be a relative. We have also had heat-stable alkaline phosphatase, α-fetoprotein, and chorionic gonadotropin. I would like to ask Dr. Knapp whether CA 125 is better than any of these and, if the answer is yes, whether it is better than any two or three of them together.

Dr. J. Donald Woodruff, Baltimore, Maryland. Certainly the approach that Dr. Knapp has taken is the one that we needed to take to identify the presence of tumor by a noninvasive technique. However, since we are dealing with an intraperitoneal disease rather than an ovarian disease and since many other lesions involving the female pelvis will result in positive findings, namely, endocervical, endometrial, and endosalpingeal malignancies, I wonder if Dr. Knapp has used this marker for endometriosis, which is also an endoperitoneal disease, and also were other benign conditions such as salpingitis used as controls?

Dr. Alfred I. Sherman, Detroit, Michigan. I want to congratulate you on your continued effort to find and use a marker or an antigenic testing system for this disease which we would very much like to have and which would be most important.

I presume from what you said that the 35 U standard was set arbitrarily based on a large series of samples. However, what I would like to know is whether there is any correlation between the amount of tumor and the units as recorded by your system. This would be particularly important in that small group of patients who have very small residual tumors. In these cases, if the 35 U standard was set lower then there would be a greater accuracy associated with the procedure than if an arbitrary positive and negative was set at the 35 U level.

Dr. William J. LeMaire, Miami, Florida. Have you had any opportunity to determine the presence of this antigen in the tissue obtained during the second- or third-look procedures to see if levels of CA 125 might correlate with the serum levels?

Dr. Knapp (Closing). I have to agree with Dr. Averette about the question of laparoscopy versus laparotomy.

I often use laparoscopy in second-look procedures. However, I then proceed to laparotomy if the laparoscopy reveals no cancer.

Dr. Griffiths, in our department, is excellent at defining the status of cancer with laparoscopy. He can visualize the diaphragm with laparoscopy better than can be done with laparotomy. Dr. Griffiths has published his data showing the excellent results obtained with laparoscopy at second-look procedures. One of my concerns regarding laparoscopy is the inability to evaluate the retroperitoneal space and particularly the lymph nodes.

A major conclusion of this study reveals that an elevated antigen level is associated with the presence of cancer. The assay is even more critical if one is using a surgical procedure such as laparoscopy which may be less than optimal. However, the use of laparoscopy in the study does not influence the data as the finding of cancer by laparoscopy is as valid as that found by laparotomy.

CA 125 is distinct from the carcinoembryonic antigen. They do not coprecipitate. In our report in *The New England Journal of Medicine* where we monitored the course of epithelial ovarian cancer with CA 125 and carcinoembryonic antigen, serial CA 125 levels correlated with the clinical course in more than 90% of cases, while carcinoembryonic antigen correlated in only 26%.

Several other markers have been studied in epithelial ovarian cancer. To date none have demonstrated a specificity or sensitivity comparable to that of CA 125. Since CA 125 is not detectable in approximately 20% of epithelial cancers, we are investigating other monoclonal clones in an attempt to identify a complementary antibody.

I am very pleased that Dr. Woodruff addressed the question of endometriosis. We measured antigen levels in a group of patients with endometriosis and found elevated antigen levels in patients with advanced endometriosis. However, these levels were not of the same magnitude as that observed in malignancy. When the antigen is elevated, serial levels correlate with the clinical course.

We are now asking the question, can the assay be helpful in differentiating a benign from a malignant adnexal mass. I reviewed recently a paper addressing this question by Dr. Einhorn from the Karolinska Institute. Among 100 patients with pelvic masses, 13 of 14 patients with CA 125 levels >65 U/ml had a malignancy. However, elevations >35 U/ml were observed in a minority of patients with pelvic inflammatory disease, endometriosis, and dermoid cysts. The antigen is rarely elevated in Stage III borderline tumors. However, one patient with a borderline tumor who had axillary lymph node metastasis had a positive test result.

Dr. Sherman, the choice of a reference value is arbitrary. The sensitivity and specificity of the assay will vary with the selection of the reference value. A different reference value may have to be selected when the assay is used for different purposes. Based on our previous work, we defined 35 U/ml as an elevated antigen level for this study. This level will identify 80% of patients with epithelial ovarian cancer but only 1% of normal individuals. Perhaps further study of the relationship between antigen levels and tumor burden may allow us to lower the reference value in an attempt to increase the sensitivity of the assay.

An important question is why tumor is detected in some patients with antigen levels <35 U/ml. We have observed a relationship between antigen levels and tumor burden. Therefore, the explanation for an antigen level below 35 U/ml in patients with minimal cancer may be solely related to tumor burden. However, there is another possibility. We have observed histologically that the tumor is encased in fibrosis in many of these cases. We can speculate that this fibrotic shell may interfere with the shedding of antigen. If this is true, the shedding may have serious consequences in other clinical immunologic applications. For example, this fibrosis may interfere with the delivery of antibody-linked isotopes or chemotherapy to the tumor.

Pregnancy-induced hypertension: Development of a model in the pregnant primate *(Papio anubis)*

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Experiments were performed on two groups of pregnant baboons. In the experimental group, the subrenal aortic blood flow was reduced by 58% of its original value at 100 days of gestational age. In the control group, the blood flow was measured but not restricted. In the experimental group fetal death occurred in three of 12 animals following the use of a single left-flank incision to approach both the renal artery and the abdominal aorta. In the control group, pregnancies in eight of nine animals went successfully to 165 days. In the experimental group the development of hypertension, a decrease in plasma renin activity, an increase in renal resistance, an increase in serum uric acid, and the development of glomerular changes consistent with those seen in human pregnancy-induced hypertension were noted. These studies demonstrate that pregnancy-induced hypertension can be produced experimentally in a pregnant baboon, and this model should prove useful in expediting studies on the pathophysiologic features and treatment of this condition. (AM J OBSTET GYNECOL 1985;151:987-99.)

Key words: Pregnancy-induced hypertension, primate, blood flow

Pregnancy-induced hypertension, hitherto called eclamptogenic toxemia of pregnancy, is associated with significant fetal and maternal mortality and morbidity. The etiology of this disease is unknown; therefore, the treatment remains symptomatic. Although many mechanisms have been described to explain the pathophysiologic features, Page[1] has proposed that one of the precipitating causes of toxemia is fairly sudden and sustained uterine ischemia. We agree that the "trigger mechanism" for pregnancy-induced hypertension is uteroplacental ischemia, and our previous report[2] as well as that of Abitbol[3] supports this point of view. In association with hypertension, a decrease in blood volume,[4] thrombocytopenia,[5] increased activity of the sympathetic nervous system,[6] the renin-angiotensin system,[7] prostaglandin deficiency,[8] and coexistent renal abnormality[9, 10] have been implicated.

Animal experiments have been carried out mainly in subprimate species with bicornuate uteri for a variety of reasons including cost, convenience, and availability.[11] However, the logical progress to the subhuman primate model was inevitable in view of the similarity to the reproductive physiology of the female human.

The original baboon model was designed to evaluate the concept that the initiating mechanism in the pathogenesis of pregnancy-induced hypertension is uteroplacental ischemia. The development of the model took 8 years because of the relative infertility of the animals following transection of the ovarian vessels. Inducing such ischemia during pregnancy would have the obvious advantage of expediting our investigative program, and the application of such a model to the development and evaluation of new methods of treatment is obvious. The present experiments were designed to test the authenticity of this modification of the original model.

Material and methods

Experiments were performed on two groups of pregnant baboons *(Papio anubis)*. They were obtained at 80 days of gestational age and were housed in the newly renovated animal care facility at the University of South Florida. They received identical diets and were housed in an identical environment during the entire experimental period. All of the baboons were sedated with intramuscular injections of ketamine hydrochloride (Ketaset) in a dose of 5 mg/kg of body weight and anesthetized with intravenous sodium pentobarbital. The animals were fasted 18 hours prior to the experiment, but water was given freely. Strict aseptic and antiseptic techniques were followed during all surgical procedures. All physiologic measurements were obtained after the animal was placed in the left lateral recumbent position for at least a 30-minute period.

Experimental group (group 1). This group consisted

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of 14 baboons with a body weight of 14.3 ± 0.6 kg (mean ± SE). At the gestational age of approximately 100 days, a left paralumbar incision was made and the abdominal aorta was exposed. An electromagnetic flow probe (acute) of appropriate size was placed around the aorta, below the renal level, for the measurement of aortic blood flow. A urethral dilator (Fr. 9 to 10) of approximately half the diameter of the abdominal aorta was placed parallel to the aorta and below the level of the renal arteries. This was tied securely with No. 2 silk, and then the urethral dilator was gently removed. This allowed a standard reduction in the subrenal aortic blood flow over a prolonged period of time. After removal of the urethral dilator the blood flow was measured again below the occlusion and this resulted in a 55% to 60% reduction in the aortic blood flow. Once this level was achieved the animal was observed closely for an additional 30 minutes, and then the incision was closed and the animal was returned to its cage.

Control group (group 2). This group consisted of nine pregnant baboons with a body weight of 14.6 ± 0.3 kg (mean ± SE). These were treated in exactly the same way as in group 1 except that they underwent a "sham" operation, in which the aortic blood flow was measured but not restricted.

Several parameters were used to evaluate the effect of the experimental technique on pregnancy outcome. Animals were evaluated at 100, 130, 145, and 165 days of gestational age. All fetuses were delivered by cesarean section at 165 days.

Hemodynamic studies. At 100 and 165 days of pregnancy aortic pressure was measured by inserting a polyethylene catheter into the femoral artery through a "cutdown" in the groin. The catheter was advanced into the abdominal aorta, above the renal level, so that the tip came to rest above the silk ligature in the experimental group. A Statham pressure transducer and a Sanborn recorder were used for the continuous monitoring of the arterial pressure. The heart rate was calculated from the arterial pulse tracings. In addition Arteriosonde Model 1022 (Roche) was used for the serial measurement of blood pressure at 100, 130, 145, and 165 days of pregnancy. A femoral vein was also cannulated and the catheter advanced into the vena cava. This was used for the purposes of saline solution administration and blood withdrawal.

The left renal artery was exposed extraperitoneally by means of a paralumbar incision, and the flow was measured by means of an electromagnetic flowmeter (Model 501, North Carolina Medical Electronics) and a corresponding probe. This was done at 100 days of gestational age and at the time of cesarean section. Renal artery pressure was measured as renal orifice pressure and renal vascular resistance was calculated from directly measured renal blood flow. Aortic blood flow was measured at similar times via the same surgical approach previously described.

Plasma volume was measured by the dye-dilution technique with Evans blue (T-1824),[12] and the blood volume was calculated with the use of the simultaneously measured hematocrit. After a control blood sample was obtained in heparinized tubes, 2 ml of the dye (22.6 mg in 5 ml) was injected through a peripheral vein and the blood samples were drawn at 10- and 20-minute intervals from the opposite side. The optical density of the plasma samples was read at 620 nm with a spectrophotometer (Sequoia-Turner Corp.). All the determinations were made with the subject recumbent and at rest, but not during the surgical procedure, in order to avoid lipemia and hemolysis which interfere with the optical density methods of measuring plasma dye concentration.

Hematologic and biochemical studies. Blood samples were drawn as necessary for the determination of values for the following: hematocrit, hemoglobin, platelets, leukocytes, and differential counts. These measurements were performed as previously described.[13] Serum glucose, uric acid, urea nitrogen, and creatinine were estimated by a Serometer 370 filter photometer (Mallinckrodt). These values were obtained at 100, 130, 145, and 165 days of gestational age.

Blood gas determinations. Maternal blood samples were drawn from the intravenous and intra-arterial catheters into heparinized syringes at 100 and 165 days of pregnancy. The samples of blood were immediately analyzed for pH, P_{O_2}, and P_{CO_2} with a BMS 3, MK2 blood microsystem and a PHM 72 acid-base analyzer (Radiometer, Copenhagen, Denmark) in a standard fashion.

Plasma renin activity. Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA), and tables were placed on ice. Plasma was obtained following centrifugation at 4° C and was stored frozen below −20° C until further analysis. Blood samples were collected from the inferior vena cava (below the renal level) at 100 and 165 days of pregnancy. In addition specimens were collected from the left renal and left uterine veins at 165 days. Plasma renin activity was measured by radioimmunoassay (Renak) developed by Hoffmann-La Roche Inc. The reported maximum coefficients of variation for intra-assay and interassay results are 7.7% and 8.2%, respectively, with a sensitivity of 3 pg. In this method, as in all methods of measuring plasma renin activity, renin is not measured directly but is allowed to react with endogenous plasma substrate to generate angiotensin I, which is then quantitated by radioimmunoassay.

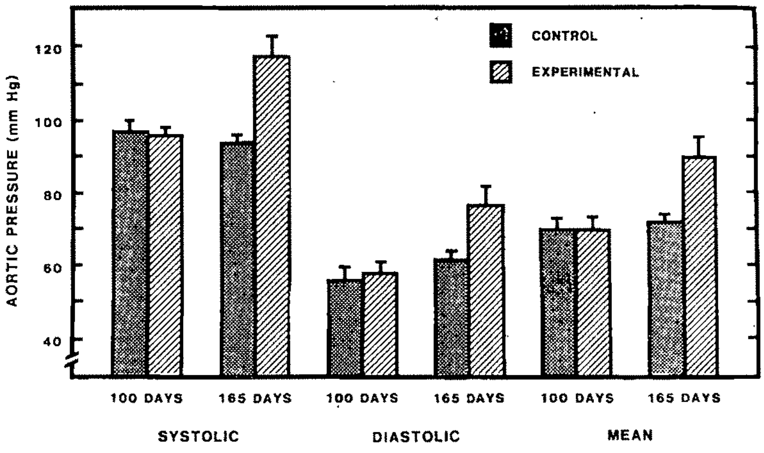

Fig. 1. Systolic, diastolic, and mean aortic pressures (mean ± SE) are presented. A significant increase ($p < 0.01$) in these values occurred at 165 days of gestation in the experimental group, with no such increase being observed in the control group.

Table I. Changes in the maternal hemodynamics, maternal heart rate, and fetal heart rate at 100 and 165 days of gestation (mean ± SE)

Parameter	*Control*		*Experimental*	
	100 days	*165 days*	*100 days*	*165 days*
Renal artery flow (ml/min)	121.7 ± 5.2	125.7 ± 10.1	144.4 ± 13.1	135.0 ± 14.4
Renal resistance (dynes-sec/cm^5 × 100)	435.6 ± 44.2	477.2 ± 43.6	410.5 ± 46.7	570.9 ± 54.9
Aortic blood flow (ml/min)	412.1 ± 23.0	490.0 ± 31.8	226.3 ± 17.1	300.0 ± 31.9
Maternal heart rate (bpm)	98.0 ± 4.5	95.0 ± 3.0	89.0 ± 4.6	105.3 ± 6.2
Fetal heart rate (bpm)	154.7 ± 5.3	153.9 ± 9.9	147.6 ± 4.9	150.6 ± 7.0

In the experimental group the aortic blood flow was measured below the subrenal constriction.

Urinalysis. Urine was obtained by catheterizing the bladder at 100, 130, 145, and 165 days of pregnancy. The levels of protein, glucose, bilirubin, ketones, and pH were determined by Labstix reagent strips (Ames Company). When protein was present it was also estimated by the turbidimetric method.[14]

Histopathologic and immunopathologic features. Needle biopsy specimens of the left kidney were obtained from pregnant baboons at 100 days of gestational age and at the time of cesarean section (165 days). Biopsy specimens obtained at 100 days of gestation served as controls.

Kidney biopsy specimens were divided into two parts. The first piece was fixed in 1.5% glutaraldehyde and 2% paraformaldehyde in 0.01 mol/L of phosphate buffer, pH 7.4. The biopsies were postfixed in 1% osmium tetroxide and embedded in epoxy resin. Sections 1 μm thick were stained with toluidine blue. Ultrathin (50 to 80 nm) sections stained in uranyl acetate were examined with a Phillips 301 electron microscope. The glomeruli, tubules, interstitium, and blood vessels were examined in detail. The second piece was frozen in liquid nitrogen. Frozen sections 6 μm thick were cut and stained with fluorescein-labeled, purified antibodies with specificity toward IgG, IgM, IgA, C3, C4, C1q, albumin, and fibrinogen. Additionally three frozen sections taken at regular intervals through each biopsy specimen were stained with hematoxylin and eosin for light microscopic examination and correlation with immunofluorescent stains.

Following cesarean section at 165 days, the placenta was examined carefully for any gross abnormalities. The weight of the fetus and placenta was recorded. The amniotic fluid was obtained by suction and the volume was measured.

Statistical analysis. The New Duncan Multiple Range Test was performed according to the method described by Steel and Torrie,[15] and, when appropriate,

Table II. Changes in hematocrit, hemoglobin, plasma volume, and blood volume at various stages of gestation in the control and the experimental group (mean ± SE)

Parameter	*Group*	*100 days*	*130 days*	*145 days*	*165 days*
Plasma volume (ml/kg)	Control	49.8 ± 2.3	58.3 ± 3.6	56.9 ± 8.8	56.6 ± 3.4
	Experimental	48.5 ± 2.0	50.8 ± 2.4	55.3 ± 2.2	55.4 ± 2.9
Blood volume (ml/kg)	Control	71.6 ± 3.1	82.6 ± 4.2	79.0 ± 9.9	79.8 ± 6.0
	Experimental	67.8 ± 2.8	75.7 ± 2.8	77.8 ± 3.2	80.8 ± 3.1
Hematocrit (%)	Control	36.5 ± 1.0	34.8 ± 1.7	34.1 ± 1.1	34.4 ± 1.1
	Experimental	33.8 ± 0.8	33.6 ± 1.1	34.6 ± 1.4	34.6 ± 1.2
Hemoglobin (gm/dl)	Control	12.0 ± 0.32	11.8 ± 0.60	11.0 ± 0.42	11.0 ± 0.62
	Experimental	12.2 ± 0.30	12.0 ± 0.40	12.1 ± 0.50	12.0 ± 0.40

Student's *t* test was also used. Probability of less than or equal to 0.05 was considered to be significant. All results are reported as mean ± SE.

Results

In the experimental group, five of the 14 animals aborted following the initial surgical procedure at 100 days of gestational age. During the first phase of the experiments, we approached the renal artery by a left paralumbar incision and the abdominal aorta by laparotomy in two animals. Both of these animals delivered within 48 hours. The surgical stress, coupled with the handling of the pregnant uterus, may have contributed to premature labor in these animals, so we elected to approach both the renal artery and the abdominal aorta with a single left paralumbar incision. Since then, pregnancies in nine of 12 animals went successfully to term and the mean values are presented in this report.

In the control group, pregnancies in eight of nine animals went successfully to term, and the mean values for these are presented.

Studies in the mother

Hemodynamic studies. Systolic, diastolic, and mean aortic pressures were measured by indwelling catheters in both groups and are presented in Fig. 1. At 100 days of gestation, the pressures from the experimental group were not significantly different from those of the control group. However, in the experimental group the systolic, diastolic, and mean aortic pressures had increased by 21.4%, 33.6%, and 28.7%, respectively, at 165 days of gestational age as compared with the baseline values obtained at 100 days of gestation ($p < 0.01$). When compared with values in the control group of similar gestational age, these values were significantly different ($p < 0.05$). In the control group, the pressures remained relatively stable during the entire experimental period.

It should be noted that we measured the blood pressure with an ultrasonic blood pressure monitor (Arteriosonde 1022, Roche) at 100, 130, 145, and 165 days of gestational age. We found these measurements to be at variance with the pressures measured directly using indwelling catheters at 100 and 165 days and concluded that, in our hands, this is not a reliable substitute for direct measurement. Therefore, the pressures obtained by the ultrasonic device are not reported here.

There was no significant change in the heart rate in both groups when compared with the baseline values, with a modest increase of 18% occurring only in the experimental group (Table I).

Renal artery flow did not change appreciably in both groups (Table I). In the experimental group the renal artery flow was 6% below the baseline value at 165 days of gestation, whereas in the control group it was 3% above the baseline value (100 days of gestation). On calculating renal resistance at 165 days of pregnancy, an increase of 9% was observed in the control group, whereas in the experimental group it was 39% ($p < 0.05$).

Aortic blood flow was measured below the renal level. At 100 days of gestation, the baseline reading in the control group was 412 ± 23 ml/min. At the time of cesarean section (165 days) repeat measurements were made and the blood flow increased to 490 ± 32 ml/min. In the experimental group, the mean value at 100 days of gestation was 539 ± 57 ml/min. Following aortic constriction the measured blood flow below the constriction was 226 ± 17 ml/min, showing a 58% reduction. Repeat measurements were made at the time of cesarean section (165 days) and the blood flow increased to 300 ± 32 ml/min below the constriction. The results are presented in Table I.

Plasma volume was measured at 100, 130, 145, and 165 days of gestational age in both the experimental and control groups. The blood volume was calculated by use of the simultaneously obtained hematocrit values. A maximum increase in plasma volume occurred at 130 days in the control group and remained stable throughout the remainder of pregnancy. In the experimental group the plasma volume expansion was delayed and reached its peak value at 145 days, after which it remained essentially the same during the rest of the pregnancy. These differences were not statistically significant and the mean values for groups 1 and 2 are presented in Table II.

Hematologic and biochemical studies. Hematocrit and he-

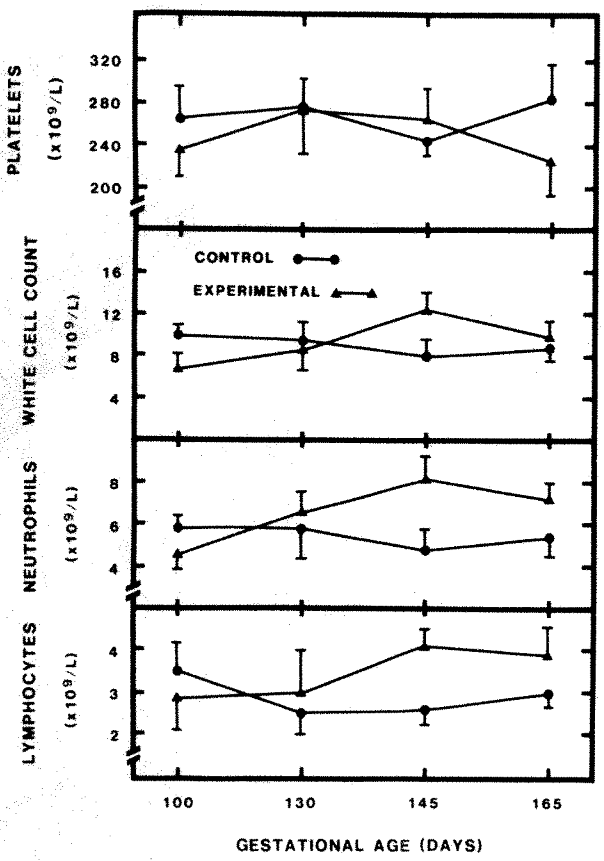

Fig. 2. Values for platelets, white blood cells, neutrophils, and lymphocytes (mean ± SE) at various stages of gestation in the two groups are shown.

moglobin values did not change significantly during the course of the study in either the experimental or the control group (Table II). In the experimental group a slight decrease in the platelet count and an increase in the white blood cell count occurred during the course of the study (Fig. 2). In the control group there was no significant change in the platelet count but a decrease in the white blood cell count was observed during the course of the experiment. The changes that occurred in the white blood cell count in both groups appeared to be a reflection of the changes in neutrophils and lymphocytes.

Serum glucose, creatinine, and urea nitrogen in both the control and experimental groups did not show statistically significant differences when compared with the baseline values obtained at 100 days of gestation. However, serum uric acid showed a tendency to increase in the experimental group and was significantly different ($p < 0.05$) at 145 days when compared with the baseline values obtained at 100 days of gestation, with no such increase being observed in the control group (Table III).

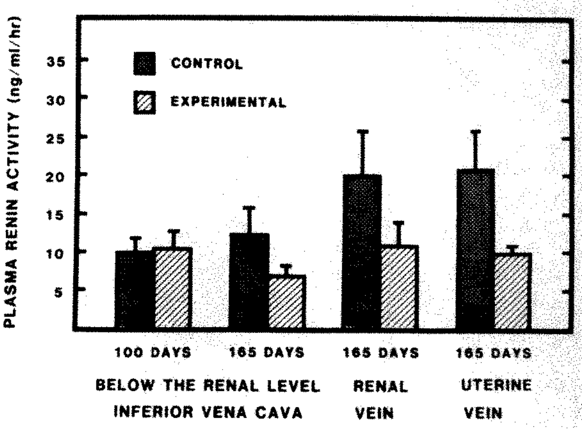

Fig. 3. Plasma renin activity from the systemic, the renal, and the uterine venous samples. A decrease in plasma renin activity was observed in these vascular beds at 165 days of gestation in the experimental group when compared with the control group. Statistical significance ($p < 0.05$) was seen only in the uterine venous blood.

Plasma renin activity. Plasma renin activity was measured from the blood samples drawn from the vena cava, below the renal level, at 100 and 165 days of gestation. The plasma renin activity levels were relatively similar in both groups at 100 days of gestation. At 165 days of pregnancy the plasma renin activity levels in the control group increased by 27% and decreased in the experimental group by 34%. Renal and uterine venous blood samples were obtained at 165 days of gestation in both groups. The measured plasma renin activity showed a decrease in both vascular beds in the experimental group when compared with the control group with a significant difference ($p < 0.05$) being observed in the uterine venous blood samples (Fig. 3).

Blood gas determinations. Arterial and venous pH, Po_2, and Pco_2 were measured in both the experimental and the control groups and the values remained within normal limits at 100 and 165 days of gestation.

Urinalysis. Urinalysis was negative for ketones, bilirubin, and blood in both groups studied. In the control group, glucose was present in only one animal, mean pH ranged from 6.7 to 7.0 at different stages of pregnancy, and none of the animals showed evidence of proteinuria. In the experimental group, urinalysis was negative for glucose in all animals. Proteinuria was present in three of the nine animals, with two animals having 30 mg/dl and the other having 100 mg/dl of protein in the urine. The mean pH ranged from 6.6 to 7.0 at different stages of pregnancy.

Histopathologic and immunopathologic features of the kidney. The kidney biopsy specimens were examined by light, fluorescent, and transmission electron microscopy.

LIGHT MICROSCOPY. Kidneys of control animals that underwent the "sham operation" were unremarkable. Glomeruli from kidneys of the experimental group of-

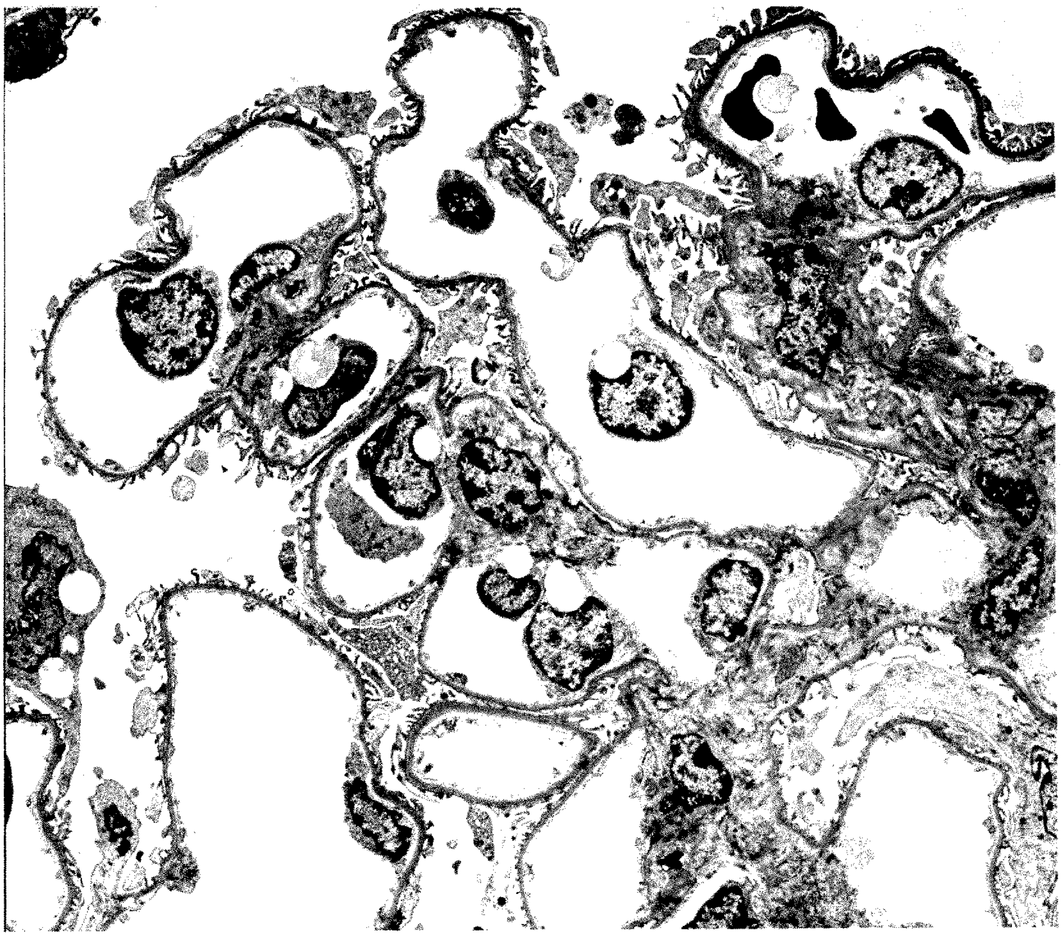

Fig. 4. Biopsy specimen of the kidney at 100 days of gestation. Electron micrograph showing portion of the glomerulus with normal architecture and widely patent capillaries. The findings were the same in the experimental and control groups. (Original magnification × 2,400.)

Table III. Variation in blood chemistry values at various stages of gestation in the control and the experimental group (mean ± SE)

Parameter	*Group*	*100 days*	*130 days*	*145 days*	*165 days*
serum urea nitrogen (mg/dl)	Control	10.4 ± 1.10	10.3 ± 0.97	8.7 ± 1.20	11.8 ± 1.6
	Experimental	8.3 ± 0.82	9.0 ± 0.70	10.1 ± 1.10	11.3 ± 0.90
Serum uric acid (mg/dl)	Control	0.70 ± 0.06	0.75 ± 0.03	0.64 ± 0.09	0.56 ± 0.07
	Experimental	0.58 ± 0.06	0.77 ± 0.06	0.87 ± 0.12	0.71 ± 0.08
Serum creatinine (mg/dl)	Control	0.33 ± 0.09	0.39 ± 0.10	0.39 ± 0.12	0.47 ± 0.10
	Experimental	0.29 ± 0.10	0.38 ± 0.11	0.48 ± 0.18	0.38 ± 0.12
Serum glucose (mg/dl)	Control	77.9 ± 6.3	83.1 ± 7.4	76.2 ± 13.0	77.3 ± 11.6
	Experimental	68.7 ± 9.0	70.2 ± 2.3	67.3 ± 6.3	65.6 ± 6.2

ten showed hypercellularity, narrowing of capillary lumina, and occasional proteinaceous casts within the tubules.

FLUORESCENT MICROSCOPY. Kidney biopsy specimens of animals in the control group showed no deposits of immunoglobulins or complement, indicating absence of immune complexes. The kidneys of the experimental group showed a varying combination of protein deposits. Granular deposits of fibrinogen in the mesangial regions was the most common finding. Five animals in the experimental group showed deposits of immunoglobulins (IgM, IgG, or IgA) in conjunction with complement (C3 or C4). Presence of these immune complexes was corroborated by electron microscopy. Two of the biopsy specimens, however, showed deposits of albumin, fibrinogen, and various immunoglobulins (IgG, IgM or IgA) without simultaneous deposition of C3 or C4. This indicates an increase in vascular per-

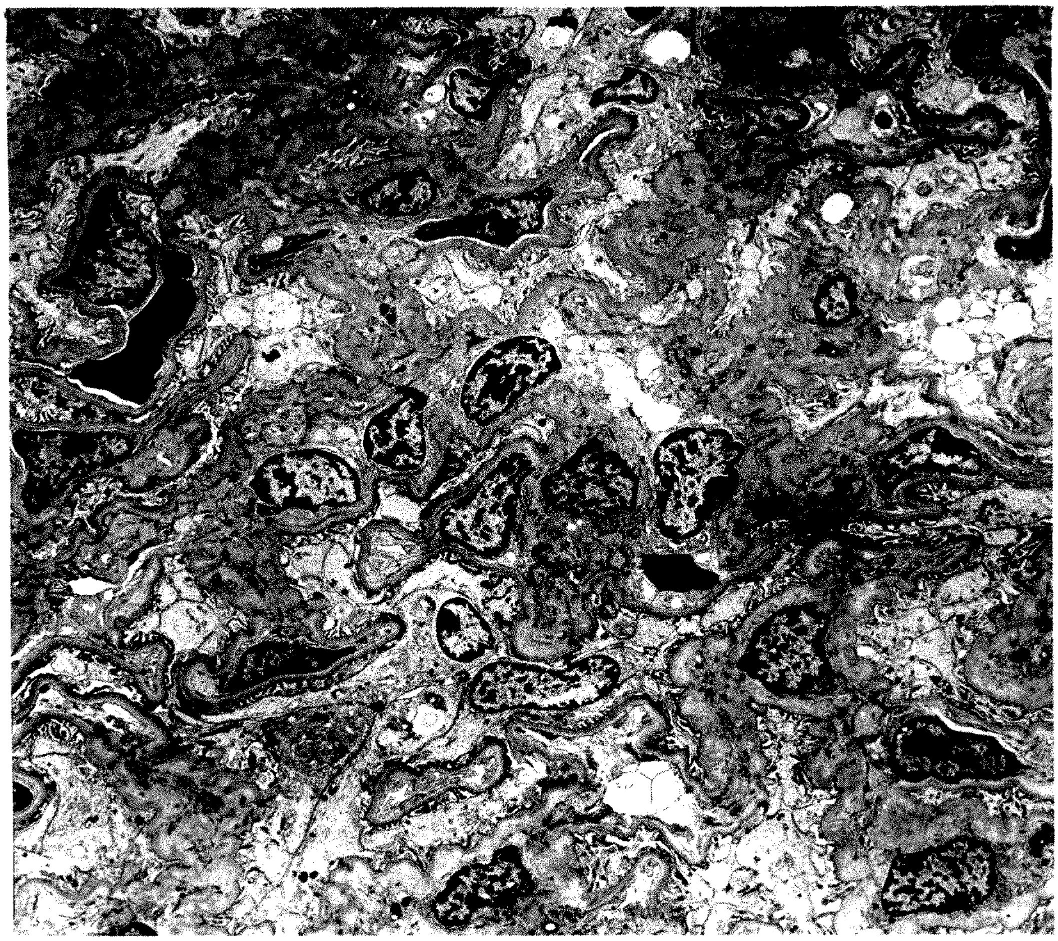

Fig. 5. Biopsy specimen of the kidney of a baboon from the experimental group at the time of cesarean section (165 days). Electron micrograph of glomerulus demonstrating widespread narrowing of the capillary lumina and an increase in both the numbers of mesangial cells and the mesangial matrix. Also the infolding of basement membrane is evident. (Original magnification × 2,400.)

meability. This theory is further corroborated by the presence of albumin, fibrinogen, and C3 deposits only in two biopsies without the presence of immunoglobulins.

ELECTRON MICROSCOPY. Detailed ultrastructural evaluation of the glomeruli present in the original needle biopsy specimens, obtained from both groups of pregnant baboons at 100 days of gestation, provided a firm basis for assessing the development of subsequent glomerular lesions observed at the time of cesarean section. In these original kidney biopsies, the glomeruli revealed widely patent capillary lumina with intact endothelial lining, unremarkable basement membranes, and normal foot processes of epithelial cells (Fig. 4). In addition, the mesangial cells and mesangial matrix were normal in appearance and distribution. In biopsy specimens obtained at the time of cesarean section, most glomeruli of the control group appeared ultrastructurally normal. However, an occasional glomerulus revealed areas with a mild increase in mesangial cells and matrix, occasional focal fusion of foot processes, and, on rare occasion, basement membrane alterations, such as rarefaction and small granular deposits of electron-dense material of undetermined origin.

The kidney biopsy specimens from the experimental group demonstrated characteristic glomerular lesions at the time of cesarean section. These included extensive narrowing of capillary lumina and a prominent increase in the area occupied by mesangial cells, their cytoplasmic processes, and mesangial matrix (Fig. 5). The cytoplasm of these processes was packed with filaments which in places formed focal densities usually anchoring at the plasma membrane. Additionally, a number of other mesangial cells contained membrane-bound dense granules. Cytoplasmic processes of mesangial cells containing filaments or granules extended into the subendothelial regions. The basement membrane showed tortuosity and frequent infoldings (Figs. 5 and 6). Focal fusion of epithelial foot processes and focal rarefaction within the zona densa of the basement membrane were seen in the glomeruli of the experimental group (Fig. 7). Electron-dense deposits, with

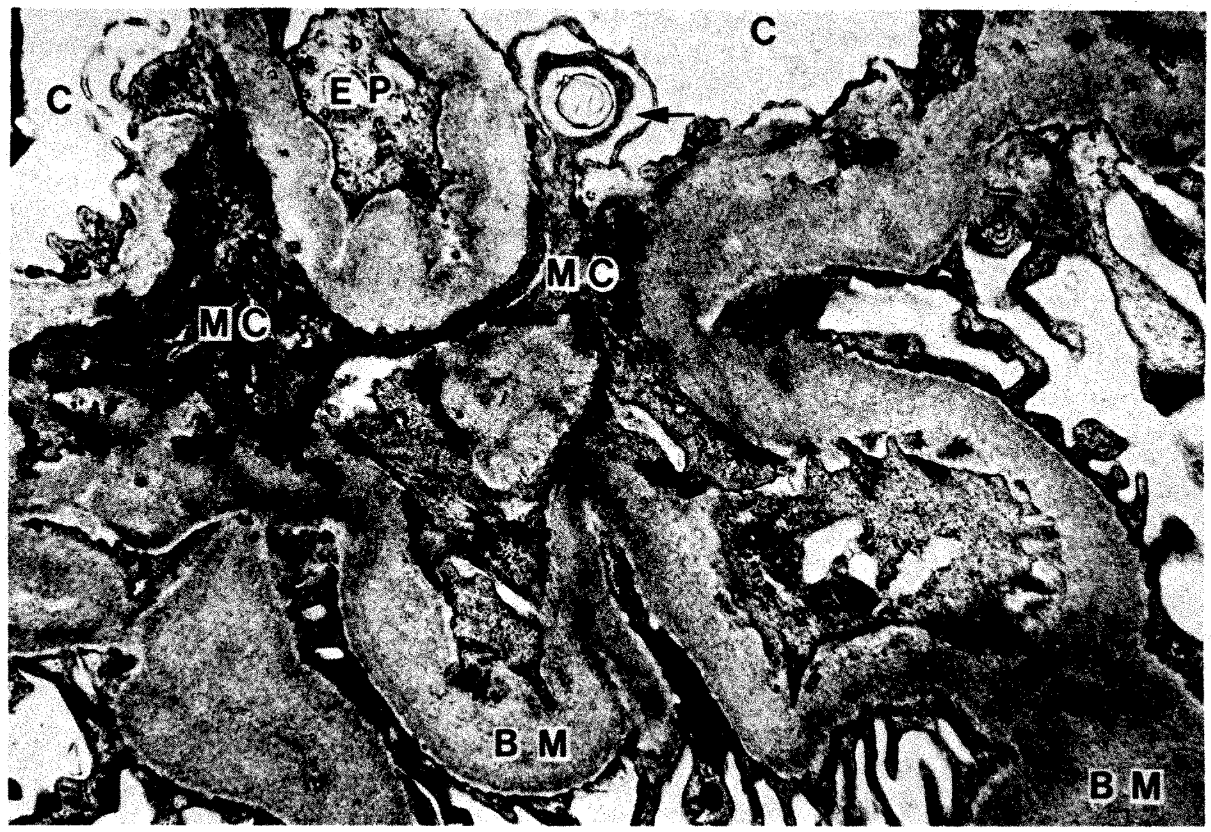

Fig. 6. Biopsy specimen of the kidney of a baboon from the experimental group at 165 days of gestation. The basement membrane *(BM)* shows marked tortuosity and the mesangial area contains cytoplasmic processes of mesangial cells *(MC)* characterized by compact, fibrillar cytoplasm. At the *arrow,* a tail of mesangial cell protrudes into a subendothelial space. *C,* Capillary lumen. (Original magnification × 16,000.)

the morphologic appearance of immune complexes, were seen within the subendothelial aspect of the basement membranes and within the mesangial matrix (Fig. 8).

Studies on the fetus. Fetal heart rate at 100 days of gestation was 155 ± 5 bpm in the control group and 148 ± 5 bpm in the experimental group. At 165 days of pregnancy the fetal heart rates were essentially the same as those at 100 days of gestation (Table I).

Each fetus was weighed following delivery by cesarean section at 165 days of gestation. In the control group the mean weight was 841 ± 38.5 gm. In the experimental group, it was 693 ± 75 gm.

In the control group the mean weight of the placenta at the time of delivery was 249 ± 20 gm, with an amniotic fluid volume of 293 ± 33 ml. In the experimental group the mean placental weight was 220 ± 25 gm, with an amniotic fluid volume of 220 ± 34.5 ml. Despite the fact that the fetal weight, placental weight, and amniotic fluid volume were considerably lower in the experimental group as compared to the control group, the differences did not reach statistical significance (Fig. 9). However, we feel that there is a trend here and that this baboon model may also be useful in the study of intrauterine growth retardation.

Comment

Naturally occurring pregnancy-induced hypertension in the subprimate species has been described.[16] The information derived from our present study clearly demonstrates that pregnancy-induced hypertension can be produced in a pregnant baboon by interference with the uteroplacental circulation. Misenhimer et al.[17] developed an experimental model of chronically impaired uterine artery blood flow in the rhesus monkey. Radioangiographic studies of uteroplacental circulation revealed markedly reduced flow in the intervillous space in some animals, but physiologic parameters were not measured. The fetal death rate after uterine flow impairment was 60%, and the technical difficulties encountered in these delicate experiments are readily appreciated by all engaged in such investigations. In our study, after we modified our surgical approach to a single paralumbar incision, the fetal death rate in the experimental group was 25%.

The development of hypertension, the decrease in

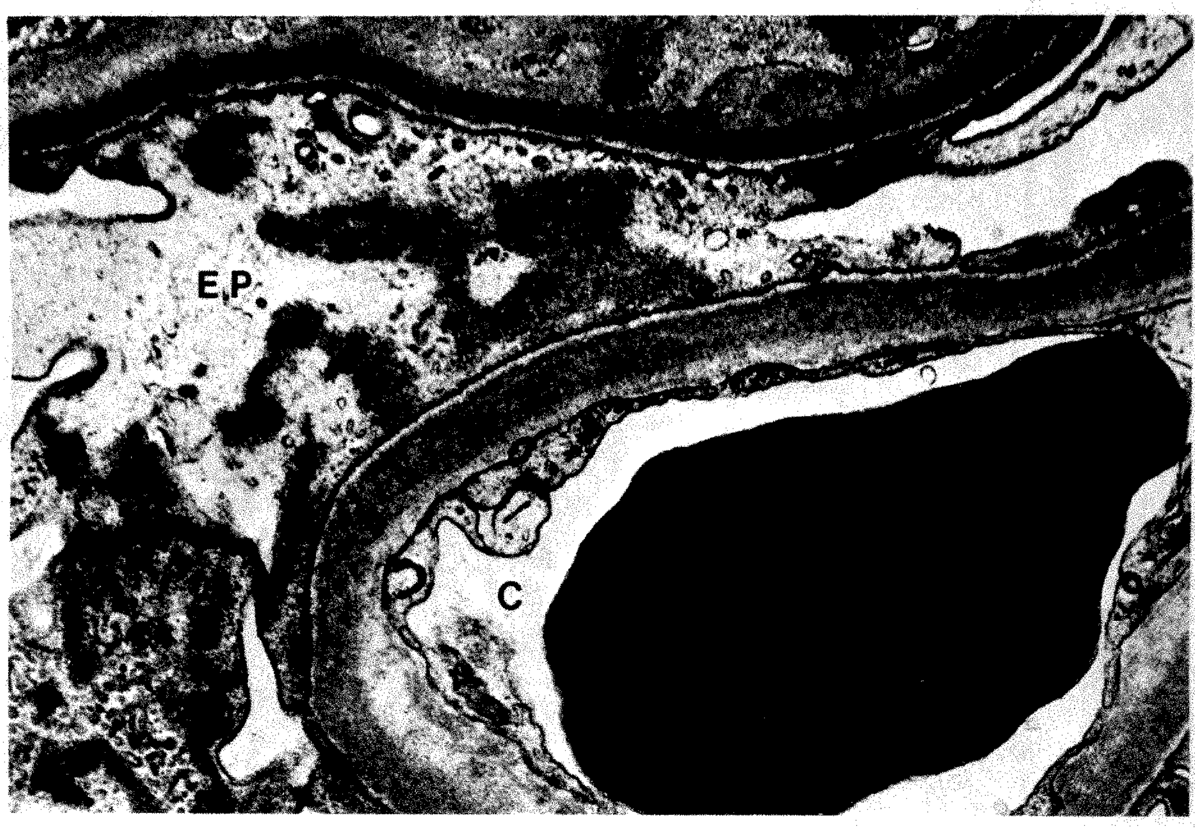

Fig. 7. Biopsy specimen of the kidney of a baboon from the experimental group at 165 days of gestation. Portion of a glomerulus showing an epithelial cell *(EP)* with focal fusion of foot processes and intracytoplasmic accumulation of electron-dense deposits. *C,* Capillary lumen. (Original magnification × 21,000.)

plasma renin activity, the increase in renal resistance, the increase in serum uric acid, and the development of typical glomerular lesions in the experimental group are consistent with human pregnancy-induced hypertension. It has been recognized that alterations occur in the renin-angiotensin-aldosterone system during pregnancy as compared with the normotensive nonpregnant state. Although opinions differ, it has generally been reported that, in contrast with normotensive human pregnancies, those complicated by pregnancy-induced hypertension are associated with lower levels of plasma renin activity, plasma renin concentration, angiotensin II, and aldosterone.[18] In our baboon study, the plasma renin activity levels measured in the systemic, renal, and uterine venous samples showed a decrease in the experimental group when compared with the control group.

It appears that the role of the renin-angiotensin system in the pregnant uterus is somewhat similar to the role it plays in the kidney in response to changes in renal perfusion pressure. Decrease in perfusion pressure leads to release of renin and production of angiotensin II. The uterus and kidney, however, are protected against the vasoconstrictor effects of angiotensin II. In the pregnant uterus angiotensin II causes release of vasodilatory prostaglandins, which in turn cause an increase in uteroplacental blood flow.[19] This increase in blood flow removes the stimulus to angiotensin production before its systemic effects are seen. This homeostatic mechanism could of course break down because of the persistence of the stimulus to angiotensin production or the failure of the protective vasodilatory system, resulting in a higher "set point" for the blood pressure "servocontrol" system.[20]

It should be remembered that responsiveness of the vascular bed to pressor substances is greater in pregnancy-induced hypertension than in normotensive pregnancy. Furthermore, Harbert and Spisso[21] have reported that the maternal placental vasculature is the primary site of uterine vascular resistance and is susceptible to adrenergic response.

In normal pregnancy, blood volume expands progressively until 30 to 34 weeks of gestation and then plateaus. Our findings in the baboon are similar to those of human pregnancy, not only in the trend toward plasma expansion but also in the absolute values expressed as milliliters per kilogram of body weight. Plasma volumes lower than those of normotensive

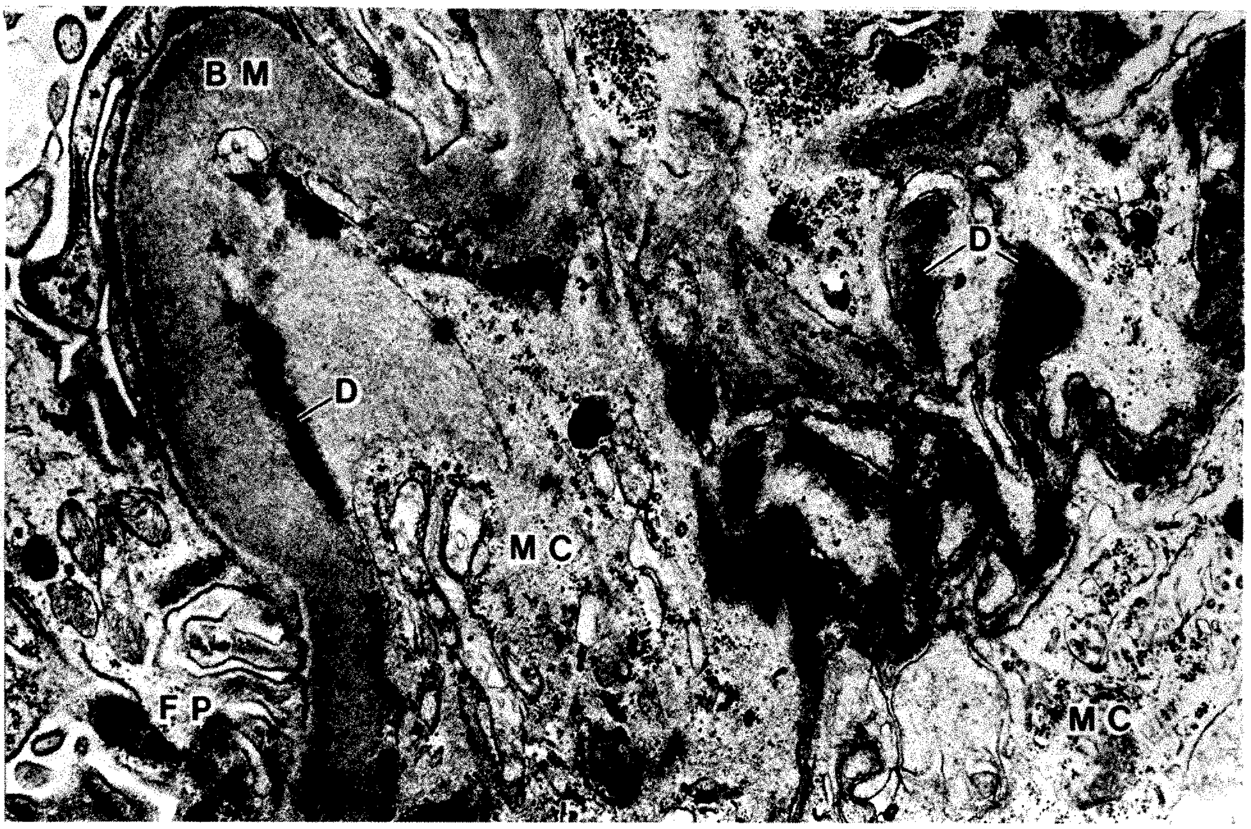

Fig. 8. Biopsy specimen of the kidney of a baboon from the experimental group at 165 days of gestation. Portion of a mesangial area showing dense deposits *(D)* in the mesangial matrix as well as in the lamina rara interna of the basement membrane *(BM)*. *FP*, Foot processes. (Original magnification × 22,500.)

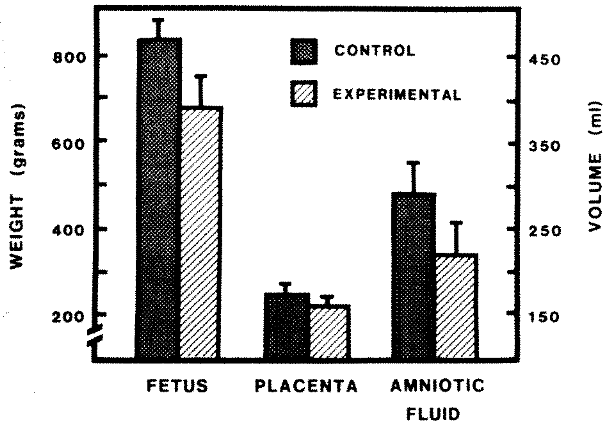

Fig. 9. Fetal weight, placental weight, and amniotic fluid volume in the two groups. Despite the fact that these values were considerably lower in the experimental group as compared with the control group, the differences did not reach statistical significance.

pregnancies have been observed in patients with mild pregnancy-induced hypertension who were delivered of small–for–gestational age infants.[4] However, the mean plasma volume in patients with mild pregnancy-induced hypertension delivered of average–for–gestational age infants was similar to that for normotensive pregnancies. In this study, the plasma volume in the experimental group did not differ significantly from that of the control group at various stages of gestation. However, the intensity and duration of hypertension observed in our experimental model may not have been sufficient to bring about the expected changes. Although we have no control over the intensity of hypertension that will develop in a given animal in the experimental group, we can control the duration of this hypertensive episode. Perhaps we should be directing our attention to this in the future and monitor more carefully the changes that occur in plasma volume.

Renal artery flow did not differ significantly between the control and experimental groups. However, renal resistance was increased and pathologic changes were observed in the experimental group, suggesting that renal function may have been compromised. The increase in serum uric acid observed in our experimental group may also be of some significance. Histopathologic evaluation of the kidney showed no remarkable changes in the control group. In contrast to this, the experimental animals had marked changes, mainly affecting the mesangial cells and matrix, thereby encroaching upon the glomerular capillary lumina. These observations are interesting in view of recent advances regarding the role of mesangial cells in glomerular function. There is evidence that a population of mesangial cells function as smooth muscle cells to regulate perfusion and filtration in response to local stimuli.[22] Our results further suggest that the above population of mesangial cells predominate in pregnancy-induced hypertension and also demonstrate characteristic interrelationships with constricted capillaries and with focal lesions of endothelium overlying mesangial regions. These observations support the concept that alterations in the contractile responsiveness of "myogenic" mesangial cells play a pathogenetic role in initiating glomerular and vascular dysfunction in pregnancy-induced hypertension.

The present study is a modification of our original baboon model for the development of pregnancy-induced hypertension and the findings confirm our previous report.[2] Animals in the experimental group developed hypertension and showed reduced plasma renin activity. Fetuses delivered by these mothers were of lower birth weight as compared with those in the control group; a similar trend was observed with regard to placental weight and amniotic fluid volume.

We feel that the present approach has a great advantage over our original primate model,[2] in that pregnancy-induced hypertension can be produced in a pregnant baboon in a shorter period of time. This will expedite studies on the progressive pathophysiologic changes that occur. However, modifications of this model should be devised, with particular attention to increasing the duration and intensity of hypertension without producing a rise in fetal or maternal mortality.

The expert technical assistance of Mr. Eric Spaziani, Mr. William Fay, Mr. Jack Strzlecki, and Mr. Edward Haller is acknowledged.

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Discussion

DR. GUY M. HARBERT, JR., Charlottesville, Virginia. Today Dr. Cavanagh has updated us on his continuing quest to develop a model in the pregnant nonhuman primate to study the pathophysiologic characteristics of pregnancy-induced hypertension. In contrast to previous methodology, this time he has partially occluded the abdominal aorta below the renal arteries by placement of a suture 3 mm in diameter that restricts blood flow to an average of 58% of control.

As suggested by Dr. Greiss in a discussion of Dr. Cavanagh's presentation before this society in 1976, he has included measurements of plasma volume in his current methodology, but he has not recorded the patterns of weight gain. Unfortunately he has continued to perform the measurements in the chronically instrumented but anesthetized preparation.

Since I am neither a nephrologist nor an electron microscopist my comments on the photomicrographs of the renal biopsy specimens obtained at term from the experimental group of animals will be limited. In both picture and in word description, they do show changes that are consistent with those reported in human gestation complicated by preeclampsia-eclampsia.

Physiologic evidence in support of Dr. Cavanagh's hypertensive model is found in the measurements of blood pressures, renal blood flow, serum uric acid concentrations, and plasma renin levels. However, there is concern that only two of the nine experimental animals developed proteinuria. There is also concern that the control and the experimental groups of animals may not be similar. The differences between the two groups for renal artery blood flow, hematocrit, and serum urea nitrogen were greater at 100 days, the beginning of the experiments, than at the termination of the studies at 165 days' gestation. Mean serum uric acid values not only were different at the beginning of the experiments (0.70 mg/dl for control versus 0.58 mg/dl for experimental animals) but also showed a complete reversal (0.56 mg/dl and 0.7 mg/dl, respectively) at term. Likewise, the preocclusion mean aortic blood flow below the renal level in the experimental group of baboons was 539 ± 57 ml/min compared with 412 ± 23 ml/min for the control animals. This differential in mean flow of 127 ml/min between groups at 100 days' gestation compares to the differential of 190 ml/min between hypertensive and control animals at term.

It must be reiterated that reduction of blood flow at a distant site per se cannot be presumed to reduce uteroplacental blood flow. Aortic blood flow increased 32.7% in the experimental group between occlusion at 100 days' gestation and term compared to an 18.9% increase for control animals. This and other compensatory mechanisms may account for the lack of statistical significance between groups for fetal and placental weights and amniotic fluid volumes.

Over the 15 years of his quest, Dr. Cavanagh has shown that certain changes comparable with or similar to those observed in the human with pregnancy-induced hypertension may be produced in the baboon. This has been done repeatedly by means of different methods and different sites to decrease blood flow directed toward the uterus. I look forward to future presentations on the interpretation and manipulation of these changes and their effect on measurements of placental perfusion. These could be more rewarding experiments than his stated plans to further modify the model with particular attention to increasing the duration and intensity of the hypertension without producing a rise in fetal-maternal mortality.

Dr. Carl J. Pauerstein, San Antonio, Texas. I wonder whether the question that you asked is a question that the design can answer. In other words, what you have here is hypertension and other changes induced by partial occlusion of the aorta. They may or may not have anything to do with pregnancy, and I wonder if it would not be appropriate to have another group, maybe nonpregnant female animals, maybe male animals, in which you induce this same degree of aortic occlusion and see what happens to the other parameters.

Dr. Fritz K. Beller, Münster, Federal Republic of Germany. This is an excellent and extensive study. Your data in the baboon demonstrate that a toxemia-like symptomatology can be reproduced in various animal species. We have refined the model of Abitbol by using a plastic shell for narrowing the aorta in the rabbit. If the shell is placed at the eighteenth day of gestation, 3 days later the animals develop hypertension and proteinuria but not weight gain. Serious kidney abnormality is histologically apparent 8 days after placement of the shell. If the animal with the shell in place becomes pregnant again immediately subsequent to the delivery, the blood pressure returns to normal but the proteinuria continues and the kidney lesion explains the loss in protein. I want to ask whether you are completely convinced that we can simulate in the animal the patho-physiologic features of pregnancy-induced hypertension.

Dr. Seymour Romney, New York, New York. I have a question and a suggestion for Dr. Cavanagh. Can you tell us what was the degree of occlusion at the ligature site at 165 days of gestation? I have a suggestion for future refinement. Would it perhaps be desirable to use a polyethylene clamp rather than a suture and also use a flowmeter on the uterine artery?

Dr Edgar Makowski, Denver, Colorado. Dr. Romney touched on the question that I wanted to ask, that is, why was an inflatable balloon not used around the aorta? After the animal was delivered, the aortic constriction can be released and the animal used as its own control.

Dr. Cavanagh (Closing). I think that many of the comments and the questions raised are very constructive and will be helpful to us in further work.

I would particularly like to thank Dr. Harbert for his relative benignity in assessing this study. I am well aware of the excellent studies that he has done in the rhesus monkey, and he certainly has impeccable credentials to critique the work in the baboon model. I was delighted to hear that he thinks that the primate model of pregnancy-induced hypertension is established.

With regard to the specific question that he raised as to the pattern of weight gain of the mothers and fetuses, we have not accurately observed that. We have been doing ultrasound studies on the fetuses, but we do not have clear answers yet. Certainly we must try to get some kind of estimate of their growth curve.

As to the pattern of weight gain of the mothers, we certainly did not weigh them too often, and that is because, as you know, they are a little difficult to handle. Now I know that people have used restraining chairs, and I know that you, Dr. Harbert, have used restraining chairs when dealing with the rhesus monkeys. When we were in St. Louis, we tried to use restraining chairs when we were doing studies on endotoxin shock, and we were not very successful. I am of the opinion that putting a primate, in which you are creating toxemia, in a restraining chair is going to raise more questions than it is going to answer. I think it is just simpler to go ahead and anesthetize them, as we are doing now, and make accurate measurements without getting bitten.

The point about the proteinuria is well taken, and I apologize for that error in your copy. In the manuscript we sent you we had mentioned only two with proteinuria, but there were actually three that demonstrated proteinuria. But why did only three demonstrate proteinuria? We really do not know the answer to that. When we were clipping the uterine arteries and transecting the ovarian vessels in the nonpregnant state in that particular model, proteinuria occurred as well as the "toxemic" glomerular changes in all of our animals. But in the pregnant animal model proteinuria occurred in only three of nine. It may be that the hypertension has to be more severe or more prolonged before proteinuria is obvious in the pregnant model. The other point that I should mention is that we did not find it practical to obtain 24-hour urine samples in these animals. Otherwise, we might have found that more than three of the nine had proteinuria. So these were spot samples, and in the future we should take steps to collect and examine all of the urine passed in a 24-hour period.

As for randomization, we did have 21 baboons, and we tried to randomize these and have them paired as best we could. But I think if we had had more animals then the results would probably have come out statistically better than they did with the same random process.

With regard to the question about aortic blood flow being increased more in the experimental than in the control group. Yes, we did notice that also, and I think it is an interesting point. I think that what probably happened was that in the experimental group the ligature loosened up to some extent and permitted more blood through that aorta. Even a silk ligature will stretch with pulsation.

Dr. Makowski and Dr. Romney questioned the type of occlusive device. Well, we did, in fact, try to use an occlusive device as described by Abitbol in his studies. We got these little cuffs, but whether we put air into the cuff or whether we put water into the cuff, the cuff deflated. Fortunately, we first tried them in other animals, so we did not lose any time in the studies. We worked out a lot of the technique on other pregnant animals before going to the baboon study. So while some people seem to be quite successful with occlusive cuffs, we have not been. I think it is a great idea to try to alter the aortic blood flow, but we found that it was not really practical to do it with a cuff, so we came up with a relatively crude method. In fact, we placed a No. 9 or 10 urethral sound, which is about half the diameter of the aorta, along the aorta. We then placed a tight ligature of No. 2 silk around both and then carefully removed the sound. This ligature then occluded the aorta by about 50%. Fifty-eight percent was actually the mean figure that came up.

Dr. Pauerstein asked if it would be better to have another control group of nonpregnant baboons ligated and studied, as we did the pregnant study group, especially with regard to the renal lesion. He is absolutely right in saying it would be better, and we should do it for the sake of completeness.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Conference on Smoking and Reproductive Health, October 15-17, 1985, San Francisco, California. Sponsored by Family Health International and other organizations. For further information contact: Dr. Michael Rosenberg, Family Health International, Research Triangle Park, NC 27709. Tel.: (919) 549-0517.

Modern Trends in Obstetrical and Gynecological Practice, June 6 and 7, 1985, Michael Reese Hospital and Medical Center, Lake Shore Dr. at 31st St., Chicago IL 60616. For further information contact: Department of Obstetrics and Gynecology, Michael Reese Hospital and Medical Center, Lake Shore Dr. at 31st St., Chicago, IL 60616. Tel.: (312) 791-4000.

Important Issues & Developments in Obstetrics and Gynecology, June 7-9, 1985, Kings Island Inn, Mason, Ohio. For further information contact: Susan Green, University of Cincinnati Medical Center, College of Medicine, Department of Obstetrics and Gynecology, 231 Bethesda Ave. (ML 526), Cincinnati, OH 45267. Tel.: (513) 872-5441.

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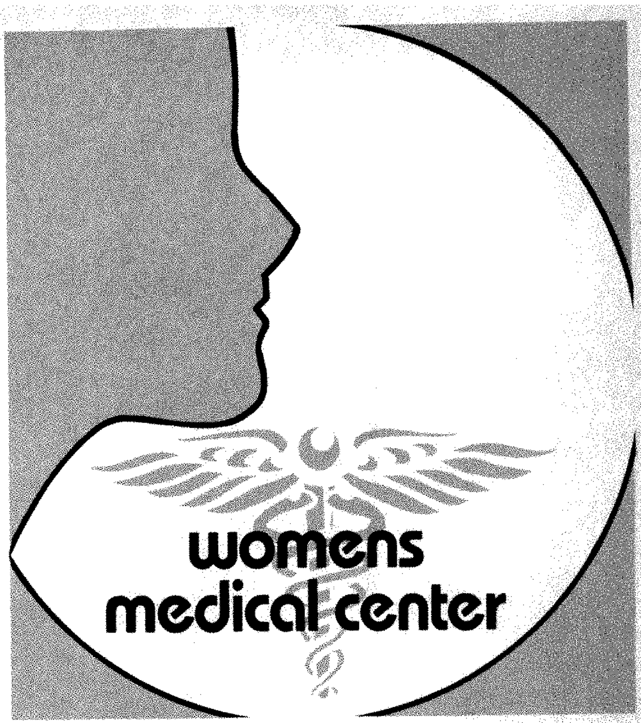

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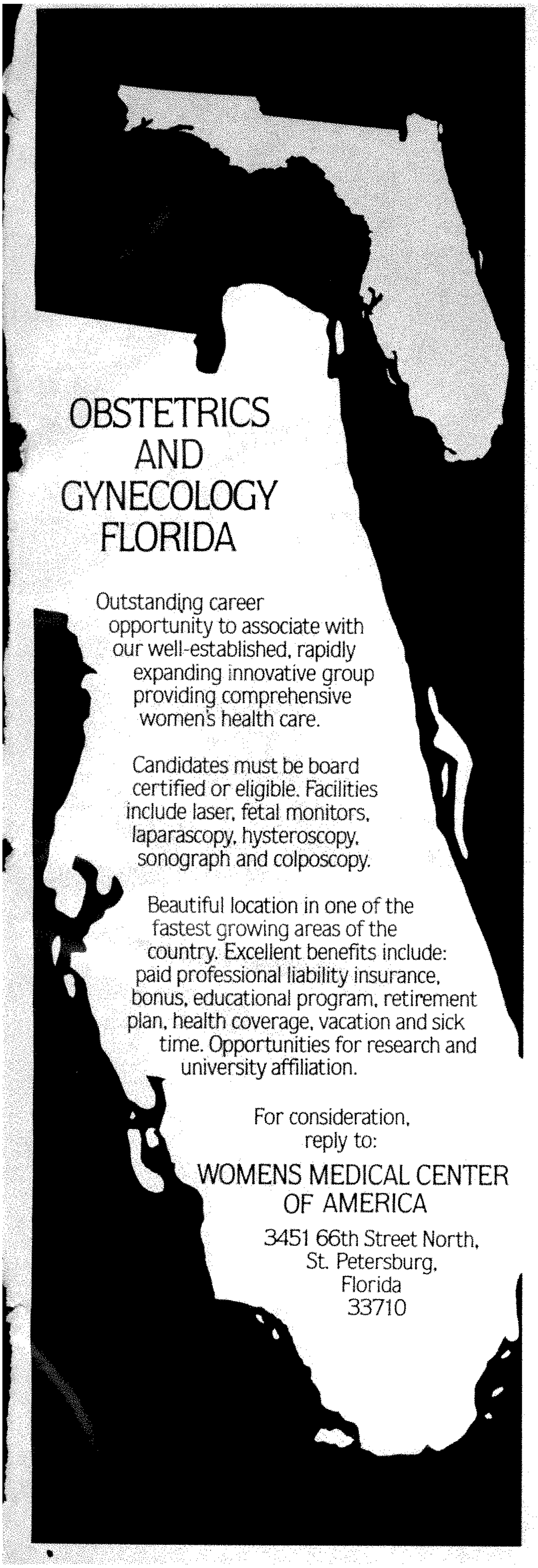

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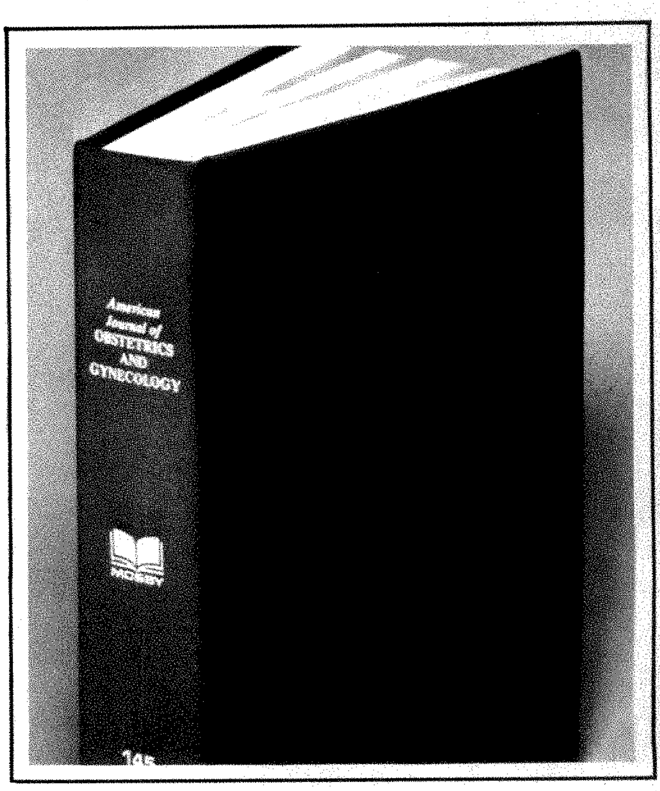

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CATIONS AND USAGE: PREVENTION OF PREGNANCY.

E-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a tive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The contraceptive product prescribed for any given patient should be that product which contains the least amount strogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

TRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1 mbophlebitis or thromboembolic disorders. 2 A past history of deep vein thrombophlebitis or thromboembo- sorders. 3 Cerebral vascular or coronary artery disease. 4 Known or suspected carcinoma of the breast. 5 wn or suspected estrogen-dependent neoplasia. 6 Undiagnosed, abnormal genital bleeding. 7 Oral contracep- tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are raindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient ld be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8 Benign or malignant liver tumor n developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

garette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. is risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in nen over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. use of oral contraceptives is associated with increased risk of several serious conditions including mboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. ctitioners prescribing oral contraceptives should be familiar with the following information relating to se risks.

ROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and nbotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great n and three in the United States have demonstrated an increased risk of fatal and nonfatal venous nboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral aceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall ss mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrova- isorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater s than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for rebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years last oral contraceptive use. A prospective study conducted in the United States found that past use of oral aceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There lso some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **ARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contracep- s been reported confirming a previously suspected association. These studies, conducted in the United , found, as expected, that the greater the number of underlying risk factors for coronary artery disease e smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the ne risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user . Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate ased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be nately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in 4 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a cardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have -fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold d risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important determining the importance of these relative risks; however, the baseline rates for various age groups given serious consideration. The importance of other predisposing conditions mentioned above in ng relative and absolute risks has not as yet been quantified. It is quite likely that the same synergistic sts, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in aceptive users persists following discontinuation of oral contraceptives and that the degree of the residual ated to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national reaction reporting systems, British investigators concluded that the risk of thromboembolism including y thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 strogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor his finding has been confirmed in the United States. Careful epidemiological studies to determine the thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. thromboembolic disease have been reported in women using these products, and they should not be d to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and s of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study t in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the y system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to er 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being trated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible er to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of ous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory s in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of der 35 with continuous use for five or more years are available, it is not possible to assess the magnitude tive risk for this younger age group. This study reports that the increased risk of circulatory disease nay persist after the pill is discontinued. Another study published at the same time confirms a previously crease of mortality in pill users from cardiovascular disease. The study concluded that the mortality with all methods of birth control is low and below that associated with childbirth, with the exception of ceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for s a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for nokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive onsmokers over 40 is higher than with any other method of contraception in that age group. The lowest y is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and otic disease associated with oral contraceptives increases with age after approximately age 30 and, for dial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of nptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive substantially increased in women age 40 and over, especially those with other risk factors. The physician patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g. ophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, senteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued ately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in traceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery associated with an increased risk of thromboembolism or prolonged immobilization. 2 OCULAR LESIONS ve been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use ontraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial lete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute ate diagnostic and therapeutic measures. 3 CARCINOMA. Long-term continuous administration of either r synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix d liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted se the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control ave reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a ases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases men without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding ral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a ral contraceptive. These products are no longer marketed. No evidence has been reported suggesting d risk of endometrial cancer in users of conventional combination or progestogen-only oral contracep- eral studies have found no increases in breast cancer in women taking oral contraceptives or estrogens y, however, while also noting no overall increased risk of breast cancer in women treated with oral tives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented ry, there is at present no confirmed evidence from human studies of an increased risk of cancer associated contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic ould be taken to rule out malignancy. Women with a strong family history of breast cancer or who have s, fibrocystic disease or abnormal mammograms should be monitored with particular care if they ral contraceptives instead of other methods of contraception. 4 HEPATIC TUMORS. Benign hepatic ve been found to be associated with the use of oral contraceptives. One study showed that oral formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5 USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6 GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7 CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8 ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9 HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10 BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11 ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12 BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1 A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2 Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3 Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4 Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5 Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6 Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7 Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8 Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9 The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10 Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea, usually the most common adverse reaction. Vomiting occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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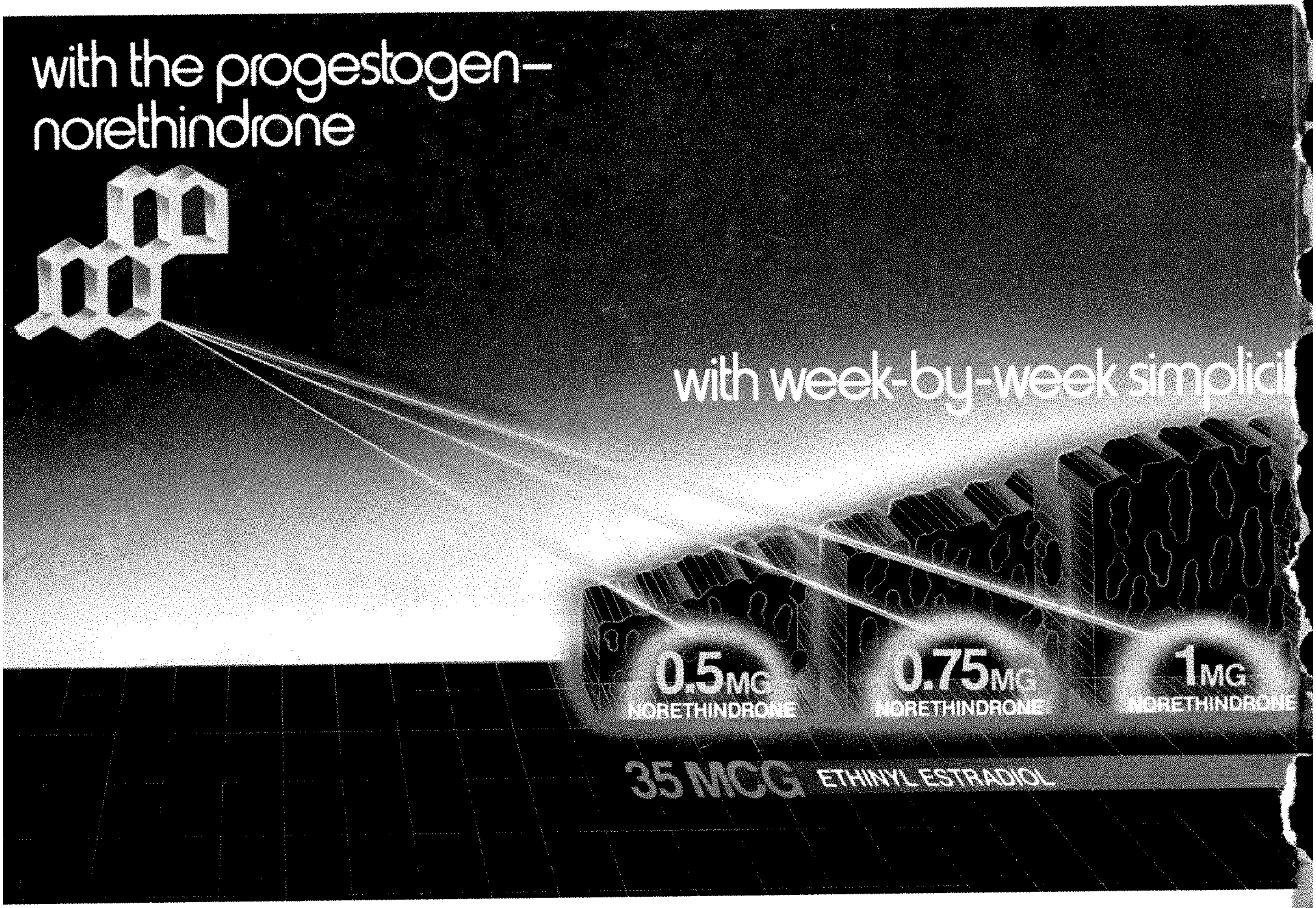
in each phase of the cycle



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21- and 28-day regim

ORTHO-NOVUM 7/7/7

TRADEMARK

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THE LOGICAL TRI-PHAS

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information on the preceeding page. ©OPC 1985 OJ-484

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